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# **LONGITUDINAL STUDY OF THE IMPACT ON HEARING FROM AGING, GENETIC BACKGROUND AND ENVIRONMENTAL EXPOSURES IN MALE TWINS**

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# Longitudinal study of the impact on hearing from aging, genetic background and environmental exposures in male twins

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

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**Stockholm 2017**



*“The roots of education are bitter, but the fruit is sweet”*

*Aristotle (384 BC – 322 BC)*

# ABSTRACT

**Background:** Hearing loss is a process that normally develops over many years, and the ability to hear high-frequency sounds is often reduced first. Age, repeated exposure to loud noise and other risk factors, diseases, medications and injury, can damage the various structures of the ear and interfere with hearing. High-frequency hearing loss is common in age-related hearing loss (ARHL) as well as in noise-induced hearing loss (NIHL). It is known that genetic and environmental factors are contributing to the phenotypic development of the hearing impairment, but the heritability of ARHL, NIHL, and tinnitus are poorly understood. Hearing impairment and/or tinnitus is assumed to be caused by multiple genes, however, no candidate gene has been found to be associated with these conditions. Environmental factors of different kinds also influence the degree of hearing loss and the combined effects of genetic and environmental influences on hearing loss are still not fully understood. This thesis aims to investigate the association between genetic and environmental influences on hearing loss and tinnitus. Twin data give a unique opportunity to explore these effects.

**Methods:** The study population was comprised of all male twins in Stockholm and Uppsala counties in 1990, aged 34–79 years at time point 1 (N = 1114) and aged 52–95 years at time point 2 (N = 583), approximately 18 years later. Clinical audiometric evaluation as well as an extensive questionnaire together with genetic analyses based on structural equation models were used to investigate the prevalence of hearing loss and the relative influence of genetic factors on ARHL (**Paper I**) with bivariate Cholesky decomposition for longitudinal analysis. The prevalence, incidence proportion and heritability of tinnitus were investigated using self-reported tinnitus complaints with co-twin analyses (**Paper II**). To investigate the environmental influence of hearing in this twin cohort, noise and solvents exposure were assessed, using the extensive questionnaire about work experience over the lifetime that were transferred to occupational work codes, and time at each job and used together with an evaluated Job-Exposure Matrix (**Paper III**). In all three papers the longitudinal approach of the study made it possible to investigate the hearing loss and tinnitus at two time points and to follow the hearing deterioration over time.

**Results:** Age was an important factor for both hearing loss (especially at high frequencies) and tinnitus prevalence. Increased prevalence of hearing loss ( $\geq 25$  dB) was seen over time. The high-frequency hearing loss (HPTA4: average of 3000, 4000, 6000, and 8000 Hz), was most affected. In the younger part of the cohort (below 66.4 age at time point 2) the prevalence of hearing loss was 69% while the older group reached a 94% prevalence at time point 2. The 18-year longitudinal hearing threshold shift, was also more pronounced for the higher frequencies, and the highest rate of change in HPTA4 was 1.3 dB per year for the oldest participants and 1 dB per year for the youngest participants. The relative genetic influences were moderate (53%–65%) for hearing loss especially at lower frequencies (PTA4: average of 500, 1000, 2000, and 4000 Hz), and were of equal magnitude at both time points. Environmental influences were of substantial importance (55%–88%) for the change



of hearing i.e. the hearing threshold shift over an 18-year follow-up period (**Paper I**). The overall prevalence of tinnitus was 13.5% at time point 1 and 33.5% at time point 2. The overall incidence proportion was 27.8%. New tinnitus cases over an 18-year follow-up period had the greatest hearing threshold shift at the higher frequencies. The relative proportion of additive genetic factors for tinnitus was 0.40 at both time point, and the influence of individual-specific environment was 0.56 to 0.61 (**Paper II**). Occupational noise exposure over the life span affected hearing at all frequencies and also exposure to occupational noise at levels between 75–85 dBA were a significant risk for hearing loss. Impulse noise in the form of firearm use at leisure times was a statistically significant risk factor for hearing loss and tinnitus at all times. Participants with pre-existing hearing loss at time point 1 had a higher threshold shift over the 18-year follow-up period (**Paper III**).

**Conclusions:** Both hearing loss and tinnitus increase with age. Genetic factors are important for hearing thresholds, but individual-specific environmental factors are more important for hearing threshold shift i.e. the rate of change for hearing over the 18-year follow-up period. A moderate genetic influence (40%) for tinnitus was shown. Pre-existing hearing loss can increase the risk of hearing impairment due to occupational noise exposure. Even low noise exposures below the current threshold limit value of 85 dBA can be a risk for noise-induced hearing loss.

**Key words:** Male twins, Longitudinal cohort study, Aging, Genetic influences, Hearing loss, Tinnitus, Pure-Tone Average, Threshold shift, Prevalence, Incidence proportion, Noise exposure, Job-Exposure Matrix, Occupational work code



# LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to by their roman numerals Paper I–III:

## **I.**

### **The Role of Genetic Factors for Hearing Deterioration Across 20 Years: A Twin Study.**

*Renata Bogo, Ahmed Farah, Kjell K. Karlsson, Nancy L. Pedersen, Magnus Svartengren and Åsa Skjönsberg. The Journals of Gerontology: MEDICAL SCIENCES, 2015, 1–7 DOI:10.1093/gerona/glu245*

## **II.**

### **Prevalence, Incidence Proportion and Heritability for Tinnitus: A Longitudinal Twin Study.**

*Renata Bogo, Ahmed Farah, Kjell K. Karlsson, Nancy L. Pedersen, Magnus Svartengren and Åsa Skjönsberg. Ear & Hearing, 2017 May/Jun;38(3):292–300 DOI:10.1097/AUD.0000000000000397.*

## **III.**

### **Influence of well-known risk factors for hearing loss in a longitudinal twin study.**

*Ann-Christin Johnson, Renata Bogo, Ahmed Farah, Kjell K. Karlsson, Per Muhr, Mattias Sjöström, Eva B. Svensson, Åsa Skjönsberg and Magnus Svartengren. International Journal of Audiology, 2017, 56:sup1, 63–73 DOI:10.1080/14992027.2016.1265157*

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## LIST OF ABBREVIATIONS

A	Additive genetic variance
A <sub>B</sub>	Genetic proportion of variance at baseline (time point 1)
A <sub>F</sub>	Genetic proportion of variance ( $a_{21} + a_{22}$ ) at follow-up (time point 2)
BE	better ear
C	Common or Shared environmental variance
CI	Confidence interval
Co-twin	Control twin within twin pair
dB	decibel
dBA	decibel A-weighting (dBA), a sound level unit
DNA	deoxyribonucleic acid
DZ	dizygotic twin pairs
E	Unique or Individual-specific environmental variance
E <sub>B</sub>	Unique or Individual-specific environmental proportion of variance at baseline (time point 1)
E <sub>F</sub>	Unique or Individual-specific environmental proportion of variance ( $e_{21}$ and $e_{22}$ ) at follow-up (time point 2)
GWAS	Genome-wide association studies
HL	Hearing level
HPTA4	pure-tone averages of the four frequencies 3000, 4000, 6000, and 8000 Hz
Hz	hertz
IP	Incidence proportion
ISO	International Organization for Standardization
JEM	Job-Exposure Matrix
MZ	Monozygotic twin pairs
PTA	pure-tone averages
PTA4	pure-tone averages of the four frequencies 500, 1000, 2000, and 4000 Hz
PTT	Pure-tone threshold
SNPs	single nucleotide polymorphism
WE	worse ear
WHO	World Health Organization









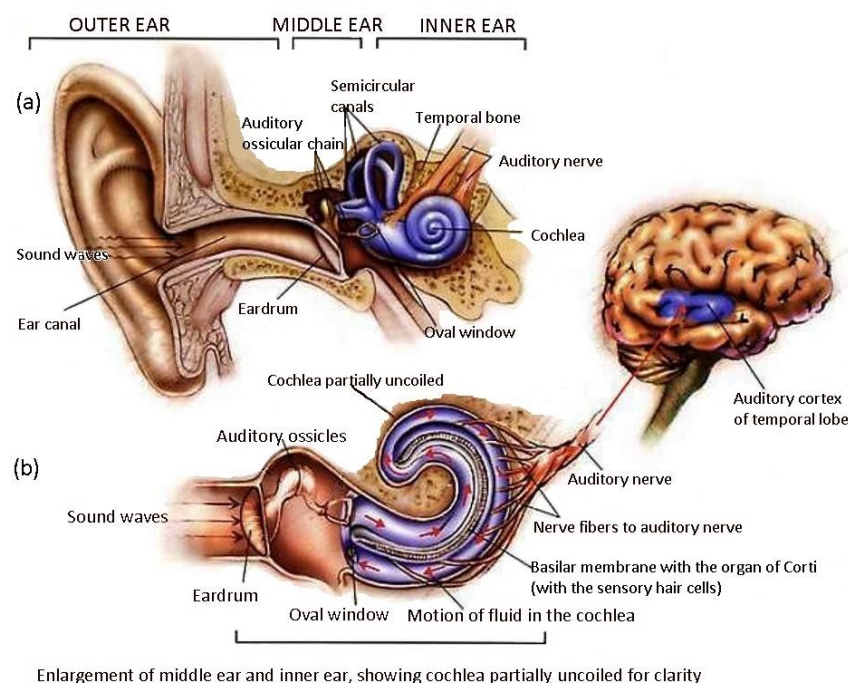
# 1 BACKGROUND

## 1.1 HEARING AND HEARING LOSS

### 1.1.1 The human cochlea

Hearing is one of our vital senses; the auditory system can locate the source of stimuli in the surroundings, and is important for distant warning and communication [1]. The human ears are paired organs, one on each side of the head, with their small size of the important inner structures well-protected within the temporal bone. Humans with normal hearing capacities can accurately localize the source and the direction of a sound, and we turn our head to look for a speaker, or observe passing vehicles at zebra crossings. Hearing and perception of sounds and speech is due to a precise sequence of events from the outer ear to the brain. The events will be shortly summarized here.

The human cochlea can detect frequencies (pitches) from about 20 to 20000 Hertz. The organ of Corti on the basilar membrane situated in the Scala Media, contains approximately 30 000 sensory cells called the hair cells in each ear [2]. The organ of Corti and the hair cells are tonotopically arranged, i.e. different parts of the organ along the basilar membrane are sensitive to different frequencies. At the base of the cochlea, near the oval window hair cells are sensitive to high frequencies, and continuing higher up towards the apex, hair cells become more sensitive to lower frequencies, an organization also known as frequency selectivity. The coiled basilar membrane is about 35 mm long [3] (see Figure 1).

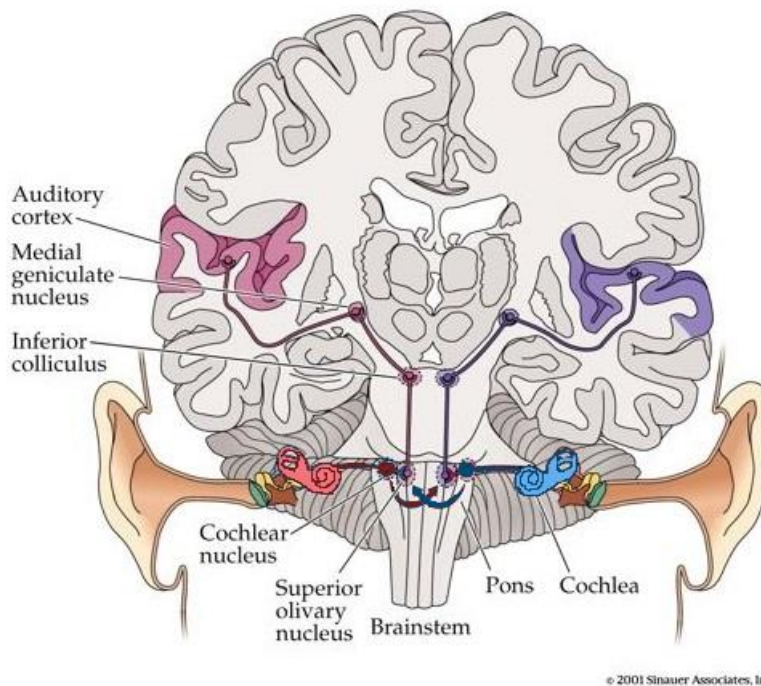


**Figure 1.** (a) The anatomy of the human outer, middle and inner ear. The cochlea is about the size of a small green pea. (b) An enlarged and schematic picture of the cochlea showing the location of the basilar membrane (35 mm long) and the organ of Corti with the sensory hair cells and their connections to the auditory nerve. Picture source: [www.murrayhearing.com](http://www.murrayhearing.com)

The tonotopic frequency representation does not exist in the cochlea alone, but even throughout the auditory nervous system [2].

### 1.1.2 Auditory system

To hear, our ears must capture the sound waves, transmit the vibrations of sound via the middle ear to the cochlea where the vibrations are translated into neural impulses, to be delivered to the auditory cortex in the brain (see Figure 2).



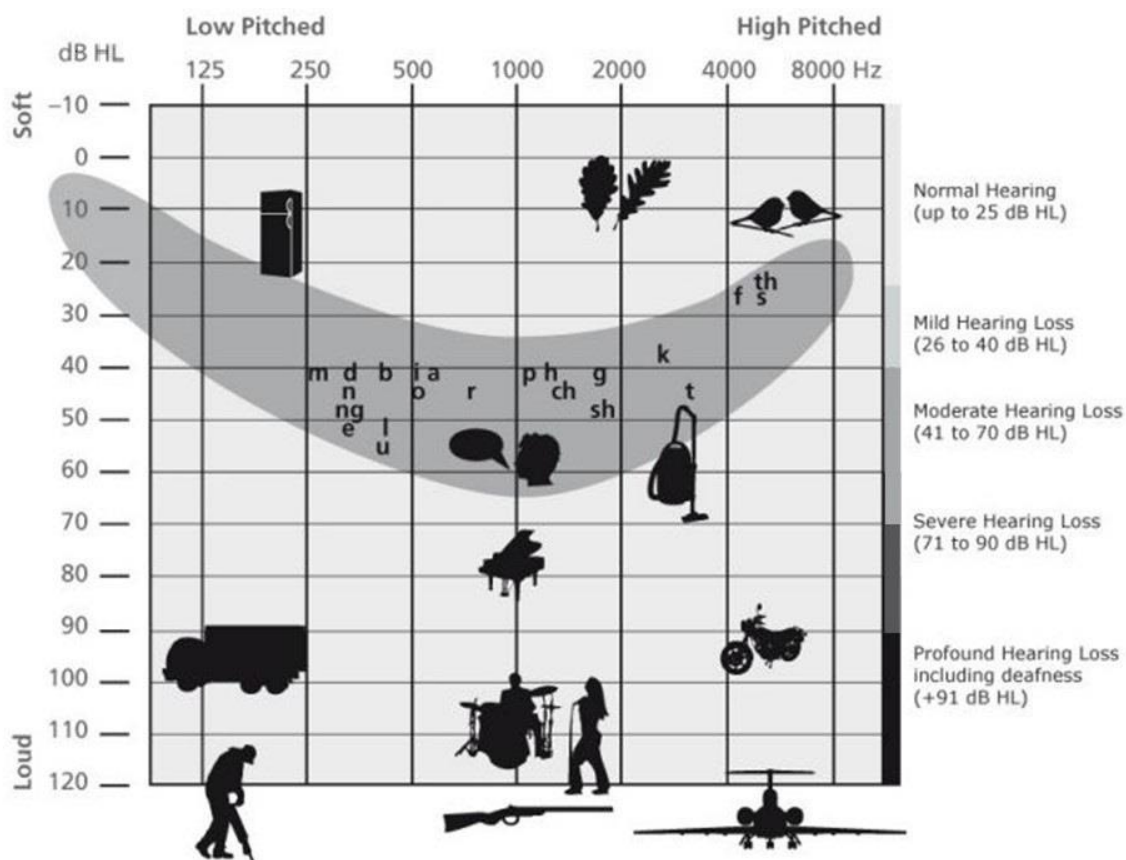
**Figure 2.** The auditory pathway from the cochlea to the auditory cortex. Picture source: [www.sinauer.com](http://www.sinauer.com)

The visible part of the ear (pinna) collects and funnels sound waves along the ear canal (resonator) to the tympanic membrane (eardrum). The middle ear contains a chain of three linked bones (auditory ossicles), as a bridge from the eardrum to the entrance of the inner ear, the oval window of the cochlea. When sound waves strike the eardrum, it starts to vibrate, and the auditory ossicle chain conducts the vibrations towards the cochlea in the inner ear. The cochlea contains the sensory organ of hearing (organ of Corti). The organ of Corti is located along the basilar membrane and contains the sensory cells (hair cells) that transform the vibrations of the basilar membrane into a neural code. The hair cells have bundles of stereocilia on their top, which respond to vibrations and converts the mechanical stimulation into a chemical signal that in turn generate nerve impulses in the auditory nerve (8<sup>th</sup> cranial nerve). These signals are then transferred via the auditory nuclei in the brainstem to the auditory center in the brain where the information of the sound is perceived and interpreted [2].

### 1.1.3 Audiogram and normal hearing

Hearing is tested, among other tests, with pure-tone threshold (PTT) audiometry using pure tones presented at different levels and frequencies, when a test-person is placed in a sound – proof booth. When the tones reach the ear canal via earphones it is called air-conducted threshold whereas bone-conducted threshold can be obtained when tones are presented through a bone-conduction vibrator located on the mastoid process [4]. The threshold of hearing level (dB HL) is the lowest level where the test-subject responds to the tones and the answer can be secure. Zero dB HL (0 dB HL) corresponds to an average sound pressure level at which a large number of young, healthy people can accurately and evenly perceive a sound. Normal hearing is indicated by individuals being able to hear soft sounds in each tested frequency and hearing thresholds being near 0 dB HL [5]. If a hearing loss is present, it is shown as an elevated threshold at each specific frequency separately. The grade of hearing loss can be defined by severity (dB HL)(see Figure 3).

In the audiogram (frequency range from 125 to 8000 Hz), the hearing thresholds form an audiogram configuration (see Figure 4).



**Figure 3.** Audiogram with Hearing loss severity from normal hearing to profound hearing loss in dB HL. Normal hearing up to 25 dB HL, Mild hearing loss 26–40 dB HL, Moderate hearing loss 41–70 dB HL, Severe hearing loss 71–90 dB HL and Profound hearing loss including deafness over 91 dB HL. The y-axes from -10 to 120 dB HL and x-axes the frequencies from 125 to 8000 Hz. Inside the audiogram, the dark gray area in which the sounds of human speech occur is called the “speech banana” includes vowels and consonants. The picture also show symbols of the common everyday sounds with its approximate softness or loudness. Picture source: [www.davidsonhearingaids.com](http://www.davidsonhearingaids.com)

#### **1.1.4 Hearing loss**

There are three main types of hearing loss: sensorineural–, conductive–, and mixed hearing loss.

#### **1.1.5 Sensorineural hearing loss**

Hearing loss arising in the cochlea or in the auditory nerve is known as sensorineural hearing loss, and with cochlear origin it can be caused by aging, acoustic trauma, noise exposure, drugs, ototoxicity, infection and other diseases, or be congenital. Sensorineural hearing loss with origin in the auditory nerve is often caused by a benign tumor of the nerve of hearing and balance (8<sup>th</sup> cranial nerve), known under the denomination as acoustic neuroma (AN) or vestibular schwannoma [6]. Acoustic neuroma occurs approximately in 2 per 10 000 cases, however undiagnosed ANs may be present in at least 0.02% of the population. A study by Lin et al., (2005) shows that 42.8% of the case subjects had an asymmetric audiometry, the other cases were symmetrical, and the size of the AN ranged from 3 to 28 mm among 688 patients [7]. A case report about progressive unilateral hearing loss and tinnitus reveals that if a tumor occurs only on one side of the nerve growing under a period of 8 years from 5 to 40 mm it then causes vertigo, migraines headaches and speech perception at 45% (by comparison a normal speech perception is over 90%) [8].

#### **1.1.6 Conductive hearing loss**

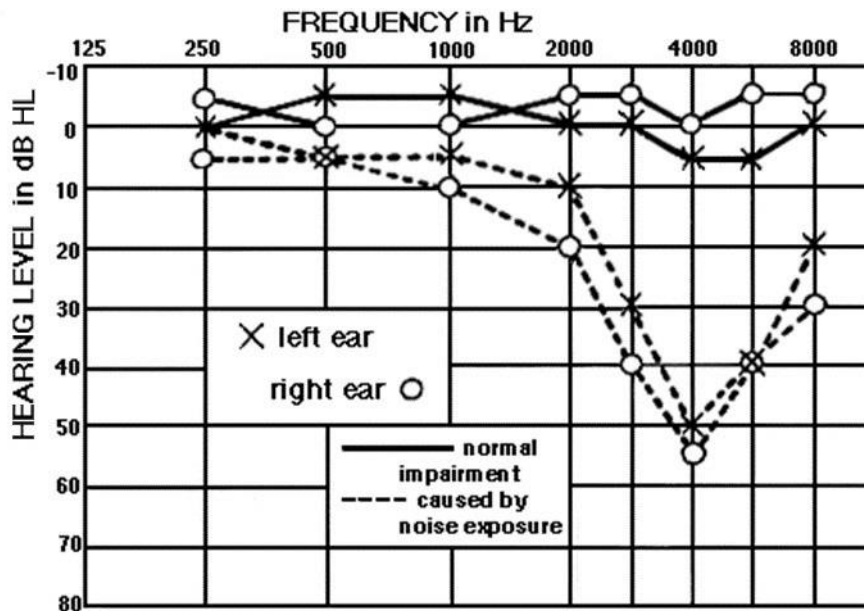
Conductive hearing loss is caused by reduced efficiency of sound waves transmission through the outer and/or middle ear. Common causes in the outer ear may be congenital atresia, cerumen (ear wax), otitis externa, exostoses, tumor of the ear canal or perforated eardrum [9]. Common cause of conductive hearing loss in the middle ear are fluid inside of the middle ear (otitis media)[10], cholesteatoma, fixation of the ossicular chain (otosclerosis), interruption of the ossicle chain, congenital malformation of the ossicles (often syndromic), middle ear tumor, and temporal bone trauma [11]. Conductive hearing loss is often reversible by surgical or pharmaceutical, depending on the nature and location of the specific cause. In general, when configuration of hearing loss on the audiogram shows a gap greater than 15 dB HL between air and bone–conduction thresholds at audiometric frequencies from 250 to 4000 Hz, the hearing loss can be classified as conductive [4].

Hearing loss can be of a mixed type combining sensorineural hearing loss with a conductive component. The audiogram configuration can reveal which frequencies involved have a conductive feature, and which frequencies involved have a sensorineural one.

#### **1.1.7 Noise–induced hearing loss**

Noise–induced hearing loss (NIHL) is one of the most common occupational disorders in Europe as well as in other part of the world [12, 13] where the prevalence of NIHL varies from 7 to 21% [14]. NIHL is the second most common cause of hearing loss after aging in middle–high income countries [15]. Noise–induced hearing damage is related to the duration (time) and intensity (decibel) of hazardous sound exposure. The noise exposure can origin

both from leisure time activities and from occupational environments [16, 17]. Configuration of hearing loss on the audiogram reveals that noise-induced hearing loss affects high frequencies more than low frequencies and affects typically the range from 3000 to 6000 Hz with a notch at 4000 Hz [18](see Figure 4). When studying war veterans it was shown that the unilateral notches were more common (69.7%) than bilateral notches (30.3%) concluded from audiometric configurations. For NIHL the threshold at 8000 Hz is usually better compared to 4000 Hz [19](see Figure 4).



**Figure 4.** Normal hearing with solid line near the 0 dB HL reference line. Circle is the symbol for the right ear and X is a symbol for the left ear. Noise-induced hearing loss with dotted line show the configuration of hearing loss with a notch at 4000 Hz on the audiogram. Picture source: [www.skeptics.com](http://www.skeptics.com).

## 1.2 AGING AND HEARING

Aging is a natural process in humans. The medical progress and lifestyle changes of this century have resulted in a longer lifespan and retained relatively good health also among the oldest old (above age 85)[20]. Ongoing population-based epidemiological studies from similar communities in the world, have collected various data to provide and report what exposures (environmental factors, lifestyle factors, medications etc.) effect the development of various diseases, disorders or other health conditions including hearing loss. The following studies below contribute to the knowledge of aging processes from an audiological perspective and highlight risk factors associated with hearing loss and/or tinnitus using either a cross-sectional or longitudinal approach (references on page 6).

It is important to follow a cohort for several decades in order to find existing differences or similarities regarding hearing impairment and/or tinnitus between generations.

Epidemiological studies provide many possibilities to focus on hearing both retrospectively, cross-sectionally and with a longitudinal setting to determine the causalities for hearing impairment.

Population-based epidemiological studies from three major continents (Australia, Europe and United States) give an insight into the association between hearing and risk factors from cohorts such as Beaver Dam, Baltimore, Framingham, Blue Mountain, Göteborg and Nord-Trøndelag. These studies show different aspects of hearing and causes for hearing impairment in general populations. The **Beaver Dam** studies investigated gender and generation differences in hearing impairment in 3000 individuals with a broad age range (21–99 years), showing the prevalence of hearing impairment ranging from 10.2% to 96.6% with increasing age. Also the influence of common risk factors such as smoking, head injury and cardiovascular diseases [21-29] was important. The **Baltimore** studies also cover a broad age range (20–95 years) showing the hearing decline from a gender perspective in approximately 1000 individuals. Hearing in men was generally worse than hearing in women up until the age of 70 [30, 31]. The **Framingham Heart Study** cohort gives an insight of how risk factors lead to the development of cardiovascular disease and its possible impact on hearing. About 2000 individuals, up until the age of 90 were investigated in each study, so that the overall prevalence of hearing loss (76% in female, 94% in male), and gender differences could be identified [32-34]. Studies from the **Blue Mountains Hearing Study** cohort focused on individuals above the age of 50 and the cohort includes between 1000 and 2000 participants. Several risk factors could be associated with hearing impairment and tinnitus, such as occupational noise exposure, history of middle ear infection, severe neck injury, dizziness, depressive symptoms and migraine [35-37]. Cohorts from Scandinavia, with homogeneous study populations comparable to the present study, are of special interest. The **Göteborg** cohort (**H 70**) included individuals born in the beginning of the 20<sup>th</sup> century. Nearly 300 individuals in an age cohort of 70-year-olds were investigated cross-sectionally and also followed a period of five years [38]. The results show that hearing problems occurred more frequently with increasing age and males reported self-assessed hearing problems more often than females. The eleven years long longitudinal pure-tone threshold decline for the H70 cohort was less for males (5 dB) than for females (15 dB), however, males had worse hearing thresholds than females [39]. Several cohorts of 70 and 75-year-olds were investigated over the years and a comparison between four age cohorts confirmed the findings of Pedersen et al., [39] that males have worse hearing thresholds than females. This comparison showed that hearing in elderly people is relatively age-stable [40]. Occupational noise exposure, however, is common. The effect of the changed exposures could not be related to the prevalence of hearing impairment even though it has varied and diminished between the 1970's and 1990's [40]. These studies also investigated the prevalence of tinnitus and it was equal for both genders, around 8% at the first time point [41], however the tinnitus prevalence had increased for males, but not for females at the follow-up [42].

The Norwegian **Nord-Trøndelag** study investigates a large population cohort (N = 50723) with a wide age-range from 20 to 101 years. The study shows pure-tone average of the hearing thresholds at 500, 1000, 2000, and 4000 Hz in the better ear as measure of hearing impairment ( $\geq 25$ dB), which comprises one fifth of the cohort. The prevalence of hearing



impairment is higher in male subjects and affects up to 32%. The gender differences are shown at high frequencies, but are most pronounced at 3000 and 4000 Hz with a maximum difference between females and males of approximately 20 dB for 55 to 74-year-old subjects [43]. The Nord-Trøndelag cohort investigated the difference between screened and unscreened subgroups, resulting in occupational and leisure noise exposure was shown to be a risk factor for hearing loss in the comparison between these groups [44].

### **1.2.1 Age-related hearing loss**

Hearing loss due to increased chronological age is often defined as presbycusis. Age-related hearing loss (ARHL) being the most common cause of acquired cochlear hearing loss is often called presbycusis synonymously [45]. ARHL is a global burden affecting at least 20–30% of the world populations over 70 years old, and 45–55% of those above 80 years old [46]. Hearing acuity and the longitudinal changes that show a gradually sloping hearing loss towards higher frequencies in the audiogram are typical for ARHL [30, 34]. The hearing loss is bilateral, progressive and symmetrical. Hearing sensitivity is commonly reduced with age (approximately over 60 years of age) and range from mild to profound deafness [45], with reduced speech understanding in noisy environments and may, therefore, include difficulties in oral communication. Untreated hearing impairment contributes to social isolation [47] and a risk for early retirement [48].

### **1.2.2 Age-related hearing loss mechanism**

The mechanism of ARHL is damage to the delicate inner ear structure and pathways, which often develops over a long period of time. Loss of sensory hair cells leads to the lack of nerve stimuli which alters the function of higher auditory centres (auditory nuclei). The damage can include a loss of both hair cells and supporting cells in the basal coil of the cochlea and the loss of neurons in the cochlea. It may also involve auditory pathway damage as well as atrophy of the Stria vascularis, and an increased stiffness of the basilar membrane [49-51]. Schuknecht has defined multiple types of presbycusis based on the study of audiometric data and the histological findings in the temporal bones of aged humans: sensory presbycusis, neural presbycusis, metabolic (strial) presbycusis, mechanical (cochlear conductive) presbycusis, and a mixed type when the four previous types of presbycusis can occur in combination [49-52]. The process of ARHL is slowly progressive over time, which means a probability of habituation and a delayed self-detection of the hearing loss, before it reaches a severe stage [47].

## **1.3 ENVIRONMENTAL AND LIFESTYLE EXPOSURES**

There is a wide range of environmental factors that influence human senses including hearing. Noise is the most well-known factor but also chemicals of different kinds can affect hearing. For review see Johnson and Morata (2010)[53].



### **1.3.1 Ototoxic agents**

Lifesaving drugs and medications can damage hearing or balance as a side effect. Medications that damage hearing are referred to as ototoxic. Ototoxic medications can enter the inner ear through the blood and cause the sensory hair cells to diminish and fade, resulting in permanent hearing loss. Ototoxic drugs include certain antibiotics (aminoglycosides)[54] and certain chemotherapy drugs such as cisplatin and carboplatin [55]. Also, quinine, which is used to treat malaria can cause hearing impairment as a side effect [56]. Drugs that are known to cause temporary hearing damage include salicylates as aspirin and also other anti-inflammatory drugs [57, 58] and diuretics as furosemide. Studies have in certain cases shown, a noise–drug interaction with more damaging effect on the hair cells [59, 60].

### **1.3.2 Solvent exposure**

Solvent exposures include toxicity to the nervous system, and other organs but solvents can also be ototoxic [53, 61, 62]. Solvent exposure usually occurs in occupational environments but people might also be exposed to solvents by accidents, abuse, in their home or at leisure time activities [63]. People are usually exposed to solvents by breathing in solvent vapors however a solvent can even be absorbed through the skin. In the work environment, people are exposed to solvents if they work with i.e. glue and adhesives, paint stripping, painting, offset printing, dry cleaning and glass fiber reinforced plastic manufacturing [62]. A study by Sliwinska–Kowalska and Davis (2012) shows that a lifetime of co–exposure to noise and solvents increases hearing loss among dockyard workers [64].

### **1.3.3 Trauma**

When a trauma occur, forceful vibrations or infection through open wounds can damage the conductive mechanism in the ear, by damage to the ear canal, eardrum and/or the ossicular chain [65]. In a worst case scenario even the auditory nerve can be damaged [66]. Major head trauma causing damage to the temporal bone or skull base can also cause hearing loss [67]. With an increasing population density and by that the associated traffic growth, the number of traffic accidents are rising followed by major injuries, including hearing damaging trauma [11].

### **1.3.4 Lifestyle**

Lifestyle factors impact our health in different ways. Consuming alcohol, drugs and tobacco, often in conjunction with a lack of physical activity and unhealthy eating, are often associated with diseases [68]. Cigarette smoking has severe negative health effects, e.g. lung cancer and heart disease. Epidemiological studies have also investigated the effect of cigarette smoking on hearing loss. The results from these studies vary, whereas, smoking as an isolated factor can cause elevated hearing thresholds. In a study from Brazil, the prevalence of hearing loss was 46.4% in a group with a joint exposure to smoking, noise and age above 40 years, whereas non-smokers, non-exposed to noise, and people between 20–40 years of age had a prevalence of hearing loss of 6.1% [69]. A study from Japan, showed no association between

hearing loss and smoking [70]. A study from Finland and Sweden did not find any increased risk between smoking and sensoryneural hearing loss [71]. In contrast, a study from Iran concluded that smoking accompanied by occupational exposure to noise may accelerate binaural hearing impairment Odds Ratio (OR) = 5.6 (95% CI 3.4–9.4)[72]. A large population-based cross-sectional study from the United Kingdom showed that current smoking status was significantly associated with hearing loss [73].

### **1.3.5 Common mechanism for extrinsic damage**

As mentioned above, noise, solvents, ototoxic drugs, trauma and disease can cause injuries to cochlear hair cells. In many cases the impaired functionality of hair cells are caused by oxygen toxicity generated by free radicals [74]. Usually the outer hair cells get damaged first and the inner hair cells may be gradually involved in addition to the cochlear neurons. The other hair cells are responsible for the sharp tuning and sensitivity of the hearing ability. Reduced outer and subsequent inner hair cell activity causes lowered activity in the auditory nerve fibers that may be followed by morphologic changes in the axons in the brainstem [75].

## **1.4 CONGENITAL AND GENETIC INFLUENCES**

### **1.4.1 Congenital hearing loss**

Congenital hearing loss is defined as hearing loss present at birth. It can be caused by one or more abnormalities in the genome, present at birth, but it can also be a genetically caused late onset hearing loss, debuting during early childhood. Congenital hearing loss can also be due to non-genetically causes such as viruses or diseases, affecting the fetus prenatally, or affecting the newborn baby at the time of birth, such as TORCH: toxoplasmosis, rubella, cytomegalovirus and herpes [76].

### **1.4.2 Hearing loss related syndromes and nonsyndromic hearing loss**

New tools and methods for genetic analysis have accelerated the discovery of genes associated with hearing impairment, which reveal that many different genes separately or in combination are responsible for auditory dysfunction. Genetic hearing loss can be subdivided into syndromic and nonsyndromic hearing loss. In different syndromes, the hearing impairment occurs together with other clinical findings usually with multiple symptoms. There are four hundred syndromes that include hearing impairment, and about 30% of all genetic hearing loss is syndromic involving more than 80 genes [77].

A heredity pattern may be autosomal dominant (risk of an offspring of a parent having an autosomal dominant gene developing the disorder is 50%); autosomal recessive (the gene that would cause the disorder is suppressed by the normal gene), there are four possible combinations from each of the parents, A/a, A/A, a/A, and a/a. The recessive trait, a, only gets expressed in the gene combination a/a. Only the offspring that inherits both mutant copies (a/a) will exhibit the trait. Overall, offspring of these two parents will face a 25% risk

of inheriting the disorder); X-linked (female inherits a defective gene on one X chromosome) the normal gene on the other X chromosome can usually compensate. As males only have one copy of the X chromosome, any defective gene is more likely to manifest into a disorder; mitochondrial (only affected mothers can pass on a disease from one generation to the next); and finally, a new mutation (hereditary: present in each of the body cells, or somatic: present only in certain cells, not in every cell in the body)[78].

About five percent of congenital hearing loss is part of a dominant syndrome, while recessive syndromic hearing loss accounts for about 20% of all types of genetic hearing loss. Less common or rare inherited patterns are X-linked and mitochondrial mutations associated with hearing impairment (see example in Table 1), for the remaining percentage of hearing loss it is still not identified.

**Table 1.** Syndromic hearing loss

Name of syndrome	Heredity pattern	Hearing Loss (HL)	Incidence frequency	Gene(s) identified	Other feature (Besides Hearing loss)	Author(s)
<b>CHARGE</b>	dominant	conductive and mild to profound HL	1/10 000 in Sweden	1	eye, heart, retardation, genital, ear	[79]
<b>DiGeorge or 22q11.2 deletion</b>	mutation	conductive and high-frequency HL	1 / 4 000 (400 reported in Sweden)	deletion on chromosome 22	any part of the body	[80]
<b>Down</b>	mutation	conductive, mixed and early ARHL	1 / 800	mutation (Trisomy 21)	chromosomal condition, mild to moderate intellectual disability	[81]
<b>Goldenhar</b>	Dominant, recessive	hearing loss	1 / 3 500 – 26 000 (20/100 000 in Sweden)	unclear	main feature development of the skull (cranium) before birth	[82]
<b>Hunter</b> ( <i>mycopolysaccharidoses Type II</i> )	X-linked recessive	conductive and sensorineural HL	1 / 170 000 (10 cases in Sweden)	1	many different parts of the body	[83]
<b>MELAS</b> ( <i>Mitochondrial Euephalopathy</i> )	mitochondrial	hearing loss	1 / 4 000	5	many part of the body, especially brain, nervous system	[84]
<b>MERRF</b> ( <i>Myoclonic epilepsy and Ragged red fibers</i> )	mitochondrial	variable HL and deafness	1/5 000	4	many part of the body	[85]
<b>Neurofibromatosis Type I</b>	dominant	can lead to progressive HL	1 / 3 000	1	tumors in different parts of the body	[86]
<b>Neurofibromatosis Type II</b>	dominant	Hearing loss, balance, tinnitus	1 / 33 000	1	tumors along in the nervous system	[87]
<b>Pendred</b>	recessive	severe to profound bilateral HL	7-8 % of congenital HL	3	thyroid gland enlargement	[88]
<b>Rett</b>	X-linked dominant	progressive sensorineural from mild degree	1/8 500	1	brain (wide range of disability)	[89]
<b>Stickler</b>	dominant	varies in degree of HL	1 / 7 500	4	facial, eye, joint problems	[90]
<b>Teacher Collins</b>	dominant	conductive HL	1 / 50 000	3	craniofacial	[91]
<b>Usher I, II, III</b>	recessive	partial or total HL	high as 1 / 6 000	12	vision impairment	[92]
<b>Waardenburg</b>	dominant	varying from normal HL to sensorineural deafness	1 / 40 000	5	pigmentation	[93]

Notes: Number of genes can have several mutations. Available from: Genetic Home Reference home page [94], Hereditary Hearing Loss home page [95] and Socialstyrelsen home page [96].

Nonsyndromic hearing impairment unrelated to other medical conditions accounts for the vast majority of genetic hearing loss (70%). Autosomal recessive inheritance is responsible for about 60% of the cases, while autosomal dominant genes cause about 33% of the hearing impaired cases. Less than two percent of the cases are caused by X-linked, mitochondrial inheritance and the rest of the genes had other inherited patterns like Y-linked chromosomal for instance [95].

The gene, *GJB2* and 19 pathogenic variants of it are estimated to be responsible for about 50% of all the recessive inherited cases of hearing loss and deafness, whereof malformation in Connexin 26 (a protein coded from *GJB2*) is the most common. Connexin 26 is an important protein in the Organ of Corti and the malformation induces cochlear development disorders, i.e. hair cell loss [97], which may cause early onset hearing loss. Mutations of two other genes (*MT-TS1* and *MT-RNR1*) have been found to cause mitochondrial nonsyndromic hearing loss and deafness, these genes account for 2% of those causes [98, 99].

### 1.4.3 Genome-wide Association Studies

There are two major approaches to identify susceptibility genes for complex disorders; linkage studies with a family-based approach and association studies, which use either family-based samples or unrelated samples. Linkage studies typically can identify rare genetic variants with large effect sizes, while association studies identify common genetic variants with small effect sizes. The majority of these studies use unrelated samples in a case-control study design [78].

A genome-wide association study (GWAS) is an approach that involves a complete set of DNA, or genomes, from many people to find genetic variations associated with a particular disease. In GWAS, researchers use two groups of participants: representative samples of individuals with the disease being studied and representative samples of individuals without the disease. Such studies are useful in finding genetic variations that contribute to common diseases, such as asthma, cancer, diabetes, heart disease and mental illnesses. The markers of genetic variation (single nucleotide polymorphisms, or SNPs) are scanned and analyzed to see if the rare variant of the SNP is associated with having the disease, and this is repeated across the genome. Once a genetic association is identified, researchers can use the information to develop better strategies to detect, treat and prevent the disease [78, 95].

GWA studies were introduced seventeen years ago as a development of studies of genetic linkage in families. Before 2013, more than 1900 GWAS reports had been published. Today in 2017, over 3000 human GWAS have examined over 1800 diseases and traits, and thousands of SNP associations have been found. In our knowledge, GWAS in nonsyndromic hearing research was studied in the 1990's, and after that dozens of studies have found over 100 SNPs associated with different hearing related traits, such as normal hearing [100], ARHL [101-104], NIHL [105, 106] and cisplatin induced ototoxicity [107].

#### **1.4.4 Genetic and environmental influences: Twin and family studies**

Twins, especially identical twins, fascinate not only the general public, but also the curiosity of researchers as a tool to explore the relative importance of genetics and the environment for a specific outcome. To aid this research, numerous twin registries around the world are being used in order to provide the possibilities to explore genetic and environmental influences for numerous traits and diseases [78].

The question asked is how much do genes and environment individualize our lives. Twin and family studies show that both genes and environment influence human traits and behavior. Identical twins (MZ) share all genes, fraternal twins (DZ) and first degree relatives on average, share half the genes, as do any other siblings. This reflects the decreasing level of genetic relatedness when studying the genetic connections of a family tree. Twin research enables to separate between genetic and environmental influences without measuring genes directly [78].

Three important components can be investigated using classical twin and family design in genetically and environmentally related individuals: genetic (heritability), similar family influence (shared environment) and unique (non-shared) environment. This design relies on statistical analyses of differences in identical and fraternal twins to decompose the variation between them into these three sources: additive genetic (A), common environment (C), and unique environment (E)[78].

Twin and family studies make it possible to study the relative importance between genetic predisposition and environmental factors in various diseases or different traits [108]. For some traits, individual differences are largely due to genetic variance (intelligence 70%, personality 40% or ADHD 45–90%), but for some abilities, like problem solving, the genetic factors seem to lose importance as one gets older [109].

Twin research is also used to study the hearing, hearing loss, tinnitus through family and classical twin design [110]. Twin studies have higher heritability estimates than family studies, they estimate the impact of shared environment and are not confounded by cohort differences. Studies investigating different hearing related traits have shown that heritability estimates vary widely, depending on which design the study used, a family design or a more common classical twin design comparing MZ and DZ twins, and, more importantly which measure of hearing was used in the study. Table 2 lists the study designs, traits, and shows heritability estimates, which are population-specific.

**Table 2.** Family and twin studies regarding traits and the heritability estimate

Traits	Heritability estimate	Biological relatedness	Number of participants	Age	Reference
<b>Hearing</b>	17% <sup>1</sup>	Sisters	11263 sibling pairs	20–101	[111]
	20% <sup>1</sup>	Broders			
	29% <sup>1,2a</sup>	DZ	329 male twin pairs	52–60	[112]
	67% <sup>1,2a</sup>	MZ			
	22% <sup>1,2a</sup>	Female twin pairs	217 twin pairs	63–76	[113]
	40%, 2%	Female (MZ,DZ)	866 twin pairs	70–76	[114]
	42%, 11%	Male (MZ,DZ)			
	47% <sup>1,2b,3</sup>	All twin pairs	557 male twin pairs	34–78	[115]
<b>Tinnitus</b>	52%, 28%	MZ, DZ			
	11% <sup>1</sup>	Family members	N = 11498 siblings	20–101	[116]
	14% <sup>1</sup>	Brothers			
	16% <sup>1</sup>	Sibling–sibling	981 siblings	50–75	[117]
	41%	Male	10464 twin pairs	< 40–85	[118]
<b>Noise sensitivity</b>	44%	Female			
	36%	Female and Male twin pairs	573 twin pairs	31–88	[119]

Notes: <sup>1</sup>with audiometric data, <sup>2</sup>high frequencies on <sup>a</sup>better ear or <sup>b</sup>both ears combined, <sup>3</sup>age group 65 and above

## 1.5 TINNITUS

Tinnitus is defined as notification of a sound, often of a ringing or buzzing character, but without an external sound source. Studies suggest that the experience of sound is created in the brain and can be perceived individually differing from person to person [120]. Tinnitus comes from a Latin word *tinnire* which means "to ring" [121]. Tinnitus itself is a hidden health symptom and moderate to severe tinnitus affects about 16% of individuals with

hearing loss [122]. Tinnitus and hearing loss also have a negative effect upon the quality of life of an individual [123] and cause an economic impact on the society with loss of individual productivity caused by sickness absence [124, 125] and early retirement [48].

Severe tinnitus could have an impact on sleep quality, such as difficulties to falling asleep, disturbed sleep, whereas mild tinnitus does not necessarily affect sleep. Tinnitus may vary in intensity, audibility, be nonstop or periodical, and can worsen by stress [126]. Indications have been found on intermittent tinnitus causing hormonal changes in the brain during stress [127], and tinnitus having a comorbidity with mild depression [128, 129].

### **1.5.1 Type of tinnitus and causes**

The primary cause of tinnitus is hearing loss specifically noise-induced hearing loss [130]. However not everyone having hearing loss experiences tinnitus whereas some people with normal hearing thresholds may experience severe tinnitus [131]. Hearing loss caused by old age or exposure to impulse noise as well as stress and hypertension have been associated with tinnitus [35, 37]. Head injury may cause tinnitus [66] as well as muscle pain in the neck region and temporomandibular problems [132, 133]. Certain drugs (i.e. salicylates) may cause tinnitus as a side effect [134, 135]. A study with 1147 participants, including both female and male, showed that tinnitus was more common among males than among females and tinnitus was more common in subjects with high-frequency steeply sloping audiogram than in subjects with a flat audiometric shape [136].

### **1.5.2 Tinnitus severity**

In an early paper by Klockhoff and Lindblom (1967) definition of tinnitus severity were introduced and tinnitus was described as varying from mild to severe [137]. Mild tinnitus does not affect a person's daily life or sleep quality, and is only noted when the surrounding environment is quiet. Moderate tinnitus may affect a person's daily life and/or sleep quality. Severe tinnitus does affect the person's daily life and sleep quality, which may cause stress, lack of sleep, and reduced working capacity, and in a worst case scenario lead to sick leave [124].

There is no nationwide survey on tinnitus in Sweden but Swedish and international studies have shown that the prevalence of tinnitus varies with age and in different populations. A systematic review on tinnitus prevalence by Rosing et al., (2016) shows that tinnitus prevalence in children varies from 4.7% to 46% in normal hearing children and the figures varies between 23.5% and 62.2% in children with hearing loss [138]. Tinnitus under the age of 30 has a prevalence of 20%, between the ages of 30–49 the prevalence is 20–24%, rising to 30% above the age of 50. Tinnitus is experienced more frequently with increasing age peaking at 31.4% between 60 and 69 years of age [139].



### **1.5.3 Assessment of tinnitus**

Tinnitus has no “gold standard” questionnaire, different studies use diverse definitions to grade and establish tinnitus experience. As tinnitus is a subjective condition; various instruments are used to measure the subjective burden of the patient’s experience. The choice of instrument in research and clinical studies, depends on study group or population and whether the measurements are longitudinal or at a single time point.

These instruments (see further below) result in diverse scales that can score tinnitus with the purpose to quantify the subjective reaction to tinnitus.

The oldest subjective response scale was introduced in 1960’s by Klockhoff and Lindblom (1967) defining a three–point scale to reflect the negative affect of tinnitus as described above [137].

Wilson et al., (1991) have developed a Tinnitus Reaction Questionnaire (TRQ). Twenty–six items were listed, regarding emotional issue, sleep issue, suicidal thoughts, depression, effects on lifestyle, general well–being, etc. The score is between 0–104 [140].

The Tinnitus Handicap Inventory (THI) was developed in 1996 by Newman and colleagues. The questionnaire includes 25 questions to determine the degree of distress suffered on a functional scale, an emotional scale and a catastrophic scale. A total score ranges from 0 to 100 and individuals reaching a score that is higher than 54 are recommended to seek professional help [141]. THI is widely used in research and clinical trials to determine the effectiveness of a given therapy.

The Tinnitus Functional Index (TFI) was reported by Henry et al., (2016). It is a 25 item TFI score ranging from 0 to 100 and defining tinnitus from not a problem to a very big problem [142].

### **1.5.4 Treatment of tinnitus**

Many kinds of tinnitus treatment are available, and appears to increase in number [143]. Hearing aid or a cochlear implant may benefit 90% of all tinnitus patients who have hearing loss as well [144], unfortunately there are a few cases that got tinnitus after cochlear implant surgery [145]. Individuals with normal hearing thresholds who experience tinnitus may benefit from sound stimulation devices [146].

The purpose of several treatments is that the brain could get accustomed to tinnitus. Tinnitus retraining therapy (TRT) by Jastreboff was a breakthrough in tinnitus treatment. TRT is long–term and consists of two parts; information and audio therapy [147].

Cognitive behavioral therapy (CBT) is a method that addresses tinnitus problems, not the sound itself. Its goal is to reduce tinnitus and increase a sense of control [148, 149].

A rather rare surgical treatment includes deep–brain stimulation to decrease the perception of tinnitus in patients with major depression [150-152].

### **1.5.5 Genetic and Environmental influences of tinnitus**

Are both genetic and environmental factors important to develop tinnitus? Several studies tried to answer that question. In a European multicenter study involving 198 families, results showed that the familial effect for tinnitus was 15% and that environmental factors were responsible for 85% of the influences [117]. Kvestad and colleagues (2010) found in their population-based study, that about 11% of the Norwegian family members had tinnitus caused by genetic influences, and the remaining 89% could be explained as caused by environmental factors [116]. A recent study by Maas and colleagues (2017) from the Swedish Twin Registry showed that self-reported tinnitus could be explained by genetic effects up to 44% for females and 41% for males, thus tinnitus has an underlying genetic background with moderate heritability [118].

## **1.6 TYPES OF STUDIES**

Various types of epidemiological studies provide information and knowledge on determining factors for diseases and prognosis which is essential for professionals and caregivers.

Epidemiological studies are not about individuals, they are always about groups, communities, or populations [153-155]. There are different study designs within epidemiology; observational studies to observe what people are doing and experimental studies to see if any intervention may be appropriate. In epidemiology, disease is distributed within a group [156].

Epidemiological studies can be used to understand incidence [22, 24] prevalence [21] and to estimate risk. This can be done in different study designs such as cross-sectional studies, case-control studies, cohort studies [157] and in longitudinal studies [158].

### **1.6.1 Longitudinal and cross-sectional studies**

Longitudinal studies are a type of observational study with an experimental research design that involves repeated observations of individuals followed over different time intervals, hopefully over many decades. In longitudinal data, it is possible to study the nature of a condition or disease. The outcome can be measured as a rate, or number of new events, and it is possible to calculate incidence rate. This thesis includes a male twin cohort that was followed for over two decades, with the main focus of the study being their hearing thresholds and threshold shifts, incidence of tinnitus, lifetime and work noise exposure were studied.

A cross-sectional study uses an observational study design, reaching out at a single time point to assess who does and who does not have a condition or disease as well as the factors associated with it. The outcome measure is often the prevalence [159]. This thesis also determines the prevalence of hearing loss and the prevalence of tinnitus and apart from the exposure to leisure time noise at two different time points.

### **1.6.2 Cohort and case–control studies**

To follow a group prospectively, another observational study design, the cohort study can be used. The cohort study follows individuals over time to find out which risk factors may or may not be able to trigger the development of a disease or condition. The end point in cohort study is dividing the population in exposed or non–exposed group. The general idea is to compare effects in groups with different degree of exposure. This might be complex when many different kinds of exposure are relevant. The results are often presented as the relative risk for the effected and unaffected in the cohort. In this type of study the population usually is selected and closed. It is important that the participants in the beginning of the study remain in the study also after a period of time to minimize the lost ones at follow–up bias. Incidence data is only possible in cohort study, because of the longitudinal design. The major source of bias in cohort studies is the selection of the population [160]. A cohort study can be expensive which is due to the large number of participants needed and for the repeated tests and/or questionnaires [161]. In this thesis, a male twin cohort was observed at two time points with twenty years between the time points. At both time points, questionnaires and audiometric data were collected, and the response rate were nearly 65%, with an overall loss of 1041 individuals from the original possible study population.

In a case–control study, as in the cohort study, the study population is closed. A case–control study is a type of observational study, often used to identify factors that may contribute to a medical condition by comparing subjects who have that condition/disease (the "cases") with subjects who do not have the condition/disease but are otherwise similar (the "controls"). In a situation with rare outcomes a large number of controls will be followed regarding exposure assessment. With a case–control study it is possible to find the increased odds of getting a condition/disease using exposed vs. non–exposed as risk factors. A disadvantage with this type of study is recall bias, depending on people's memory errors and mistakes [159]. This study is retrospective, because the study starts when the outcome is known. In this thesis, cases and controls were compared within a twin pair with different outcome measures in **Paper II** and **Paper III**.

## **1.7 DATA COLLECTION METHODS**

### **1.7.1 Theoretical frame**

Questionnaires are effective ways to reach a large number of individuals [162]. It is important to decide whether a questionnaire should be paper–and–pencil or a web–based survey, considering the participants age [163], and whether or not the survey includes sensitive questions [164]. It is also important to consider the possibility of comparing it to a previous approach [115]. To increase the response rate, the combination of questionnaire, physical measurements and also reminders are needed [165].

Sweden has a long tradition of national registries, a goldmine to perform high quality epidemiologic studies. The ten-digit personal identity number that is unique for every individual can be used in register-based data collection linking different registries to each other and thus collecting different health outcomes. Register-based research is cost effective when working with large study populations, but lack of detailed information i.e. regarding exposure and confounders is a limitation for this type of data collection [166, 167].

### **1.7.2 Exposure estimations**

Estimation of exposures in epidemiological studies are always a challenge. In cross-sectional studies the measurements are either done at a single occasion or are dependent on historical data from questionnaires or records. The ideal method for assessing the occupational and environmental exposure of subjects in epidemiological studies is to measure i.e. the noise exposure during working hours and during leisure time over a longer period of time. Unfortunately, this can be difficult or even impossible to attain in humans, when the purpose is to estimate lifetime exposure including even the past [168].

In cohort studies, exposure may be measured at several time points making comparison possible. Actual exposure measurements are often not possible in large population studies. Thus, the exposure estimates are often based on questionnaire data. Prospective exposure estimates (before the effect has taken place) are less biased. To increase the validity of such exposure data the structure of the questionnaire is important [169, 170].

### **1.7.3 Job-Exposure Matrix**

It is common to work in different occupations and to perform different work tasks during the working lifetime. Different occupations involve various exposures, such as physical and mental workload or chemical exposures. These workloads in small amounts or in short periods may be harmless, but in large amounts and over a long period of exposure time the workload can be hazardous to health. To handle these scenarios different exposure matrixes have been developed. A job-exposure matrix (JEM) is an exposure estimation technique, which can be used as a surrogate to exposure measurements. A JEM will provide information about different exposures connected to different occupations. The JEM is based on actual exposure measurement from many different sources or register, often connected to national or international occupational coding systems. JEMs have been used in epidemiological studies in many different areas, for example for lower back pain [171], for chemical exposure [172], for radiation exposure [173] and for magnetic field exposure [174]. An advantage using JEM is that they are not affected by bias from individual outcome. They are created once and possible to use at low costs. There is however a limitation regarding precision.

A very important issue in hearing research is occupational noise exposure [175]. There are regulations in most of the industrialized countries to control noise levels at work, due to the risk of hearing loss and of tinnitus [18]. The Swedish noise regulation includes two action levels; when the average noise level (8 hours per day) exceeds 80 dBA the employee is entitled to receive information about the risk of hearing loss and hearing protection should be

provided to employees if wanted. At average noise levels  $\geq 85$  dBA, which is the threshold limit value, hearing protection is mandatory and the employer is obligated to have a hearing protection program at the work place [176]. Hearing conservation programs are crucial to preserve a good hearing [177, 178].

A Swedish JEM with focus on occupational noise exposure was developed by Sjöström et al., (2013)[179]. This JEM was based on occupational work codes, according to the Nordic Occupational Classification system (NYK) consisting of a 3-digit code (NYK-85/90) [180]. The occupational classifications were made by at least two experienced occupational hygienists individually and then compared and discussed with a third expert in case of discrepancies. This JEM included occupational noise exposure levels in different occupations based on 569 measurements in Swedish work places, between 1970–2004, from 129 unique occupational codes (according to NYK–85/90) belonging to 321 job families. Three occupational noise level intervals were created, less than 75 dBA classified as low exposure, between 75 and 85 dBA as moderate and more than 85 dBA classified as high occupational exposure to noise. The noise exposure information is mostly based on noise measurements from occupational medicine clinics, occupational health services and at different workplaces on 8-hour average exposure. The risk of peak level (impulse noise) exposure was also classified. The allocations were made for each 5-year interval between 1970 and 2004. The JEM was validated using statistical methods as well as the consensus of three different occupational hygienists [179]. This JEM has been used in a study by Selander et al., (2016), who found an association between hearing dysfunction among children when the mother was working full-time during pregnancy in occupations with a noise exposure of more than 85 dBA [181].

## **2 AIMS OF THE THESIS**

In the background above, the following lack of knowledge was identified:

The relative scarce use of twin studies in hearing research means that more knowledge about the relative impact of genetic and environmental factors on hearing loss can be achieved especially when applying a longitudinal approach design.

Large population studies with cross-sectional measurements at different times, have given information about the influence of different factors such as age and the development of hearing loss prevalence at a point in time. However, these studies only follow the same individuals for a short period. The availability of longitudinal data, longer than two decades are lacking, which gives the opportunity to explore hearing threshold changes over time more than just a decade on an individual level.

Measurements of long exposure time to noise and other factors are always a challenge but the possibility to use repeated questionnaires and validated JEMs give possibilities of better exposure estimates. A unique opportunity was to apply the Swedish JEM on longitudinal data from a twin cohort and estimate the lifetime noise impact on hearing and the rate of hearing loss.

Using male, identical and fraternal twin pairs, who could contribute with genetic background on environmental measures and the interplay between genetic and environmental influences on hearing will increase the knowledge of the heritability of hearing loss. Longitudinal data on the incidence of tinnitus and heritability of tinnitus is lacking in the general population.

The main aim of this thesis was to explore the impact of genetic and environmental factors on hearing loss in a homogenous male twin cohort with a longitudinal approach.

### **2.1 SPECIFIC AIMS**

The following aims were addressed:

#### **Paper I**

To explore how aging and the genetic influence affect the hearing thresholds at different frequencies and the longitudinal threshold shifts in a male twin cohort. The hypothesis was that there should be some genetic influences on the age-related hearing loss, that age would affect the prevalence's of hearing loss differently for low and high-frequency regions at the different time points.

## **Paper II**

To explore the effect of tinnitus on hearing thresholds and threshold shifts over two decades and to investigate the relative genetic contribution to tinnitus in a male twin cohort. The hypothesis was that hearing deterioration would be faster among participants with tinnitus and that there is an underlying role of genetic influences on tinnitus.

## **Paper III**

To study the influence of environmental risk factors on hearing loss with a special focus on the longitudinal aspect. The hypothesis was that high occupational noise level predominantly should affect the hearing threshold shift during a lifetime.

### 3 METHODOLOGICAL ASPECTS

The following materials and methods were used in all three papers.

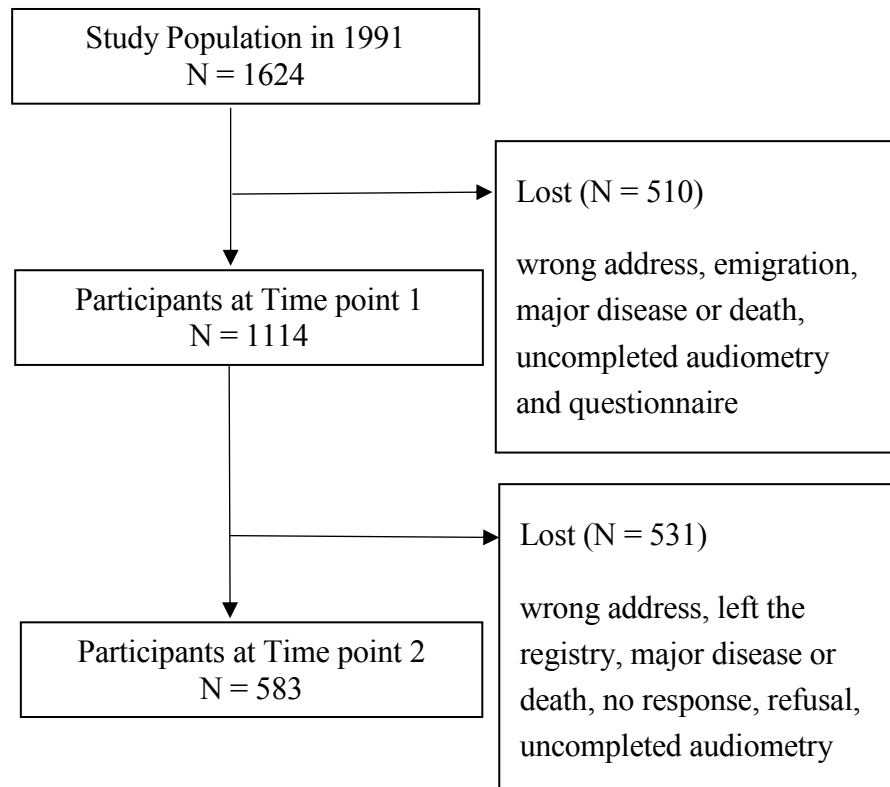
#### 3.1 SUBJECTS

The study population in 1990 was based on identification of all Swedish male twins from Stockholm and Uppsala counties, born between 1914 and 1958 and consisted of in total 1624 (812 pairs) possible participants registered in the Swedish Twin Registry, which is a population-based registry maintained by Karolinska Institutet [182]. In case one, or both brothers, chose not to participate the whole twin pair was excluded. At the first time point (year 1991 to 1995), a total of 1114 male twins (557 twin pairs, 68% response rate) finally participated in the study including audiometric and questionnaire data. The reasons for dropout are shown in Figure 5 [115]. Males included in the present study belonged to a closed cohort.

A follow-up study of the cohort started approximately 18 years later (2010–2013; the second time point) including the same measurements. Due to the age distribution (the elderly twins were born in the early 1910's and 1920's) in the cohort, 219 individuals were lost due to death. Thus the possible remaining individuals ( $N = 895$ ) were contacted by a personal letter in 2010, and asked for participation in the follow-up study at the second time. A total of three reminders were sent out by regular mail and e-mails. The responding individuals were contacted by phone to schedule an appointment. At the end of year 2013, a total of 583 individuals (239 twin pairs and 105 singletons without a participating co-twin) had completed the follow-up study including a second questionnaire and PTT audiometry. This resulted in a 65% response rate (Paper I)[183]. The different reasons for not participating during the follow-up study (from the possible participation  $N = 895$ ) were death ( $N = 31$ ), refusal ( $N = 160$ ) or unable to participate ( $N = 53$ ), and no response ( $N = 68$ ), i.e. a total of 312 individuals, thus 583 remaining participants (see Figure 5).

A total loss of 1041 individuals, more than half the population from the original study population resulted in 583 individuals, both twin pairs and singletons. The loss of twins at both time points were for a similar reason, these are: death, old age, major disease, refusal, wrong address and emigration. A decision was made for the follow-up study not to exclude the singletons, instead respect their willingness to contribute to the research for a deeper knowledge of hearing deterioration in a very old age.





**Figure 5.** Schematic flow-chart showing the process of dropouts for study population.

At the scheduled appointment, the twins had their hearing tested, completed the paper-and-pencil-based questionnaire, other measurements included blood sample, blood pressure, height and weight measurement (data not shown). In average, the procedures took approximately two hours per participants.

### 3.2 MAIN OUTCOME MEASURES

Otoscopic examination was performed on all participants before audiometric measurements. If excessive cerumen was found, it was removed before measurements.

PTT audiometry was performed in a soundproof booth with clinical audiometers and earphones (TDH39) using the ascending method, according to ISO 8253-1[184], using 5 dB steps with the lowest level detected on two out of three ascending series. Air-conduction thresholds between 125 and 8000 Hz were measured for each ear separately. The maximum stimulus levels were limited in consideration of the safety limit of the national regulations. If an individual's hearing thresholds exceeded the maximum output limit, then the unachieved hearing thresholds were registered as 99 dB HL at 125 Hz, as 109 dB HL at 250 Hz, or as 129 dB HL for the remaining frequencies according to the same procedures made by Karlsson et al., (1997)[115].

Two pure-tone averages (PTA) were used as outcome measures at both time points. The proper audiometric measurement at ten frequencies made it possible to conduct two averages, both PTA4 and HPTA4. PTA4 was an average of the frequencies 500, 1000, 2000, and 4000 Hz, an average used by the WHO to establish the severity of a hearing loss (WHO, 2010) [185]; HPTA4 was an average of the higher frequencies, including 3000, 4000, 6000, and 8000 Hz. HPTA4 were thought to be more affected by aging [42, 186] and NIHL [14, 187] than PTA4.

The HPTA4 thresholds at time point 1 for the left and the right ears were compared to assign a better (BE) and a worse ear (WE) and this assignment was then used in all analyses.

The threshold shift of hearing over time was calculated as the difference in hearing thresholds between time point 1 and time point 2 for PTA4 and HPTA4 averages as well as for each individual frequency. To evaluate hearing loss as a dichotomous variable, the value > 25 dB HL of HPTA4 at time point 1 was defined as high-frequency hearing impairment. This definition was used to divide the cohort into two groups, hearing impaired and non-hearing impaired, at time point 1 in order to compare these groups with regard to threshold shifts and exposures during the follow-up period.

### 3.3 QUESTIONNAIRE

At the first test occasion (time point 1, from 1991 to 1995), a paper-and-pencil-based survey was sent out, containing 63 questions. The questions aimed to collect information about medical background, medications, self-reported hearing and tinnitus, occupations, leisure time activities, military service, smoking etc. [115].

At the second test occasion (time point 2, from 2010 to 2013), a paper-and-pencil-based questionnaire was sent out consisting of similar but less questions (N = 43), as certain questions about medical background were not repeated and additional questions regarding tinnitus were added.

None of the questionnaires were validated, however, the same questions were presented to the same individuals at two time points. The questions were highly relevant for the addressed issues within the audiology field.

In **Paper I**, only one question from the questionnaire was used in analyses; a question regarding the twin similarity, which was asked only at the first time point: “*How similar were you and your twin brother in childhood?*” ☐ *Alike as two peas in a pod/ lika som bär* ☐ *Not more similar than siblings in general*”. This was complemented with blood analyses for mono- and dizygosity.

In **Paper II**, the focus was on the persistence and the impact of tinnitus. Questions regarding tinnitus severity from both time points were collected. “*Are you annoyed by tinnitus: buzzing or ringing in the ears?*” (“☐ yes” or “☐ no”). “*If you have tinnitus, how annoying is that?*” (“☐ Mild” or “☐ Moderate” or “☐ Severe”).

In **Paper III**, exposure information was collected from the questionnaires from both time points, using free text information about occupation, occupational affiliation, time at each workplace with noise and solvents exposure time, leisure time activities, military activities and smoking habits. Individual exposure profile were created for all participants at both time points.

### 3.4 STATISTICAL ANALYSES

In this thesis, the observed traits in addition to age were hearing impairment, hearing deterioration, tinnitus, smoking, and noise exposures.

#### *Generalized Estimating Equations (GEE) regression analysis*

In **Paper II**, the method generalized estimating equations (GEE) with binominal or multinomial distribution and logit link was used to study the effect of age on tinnitus at time point 1 and time point 2 separately and the effect of age on the combined levels of tinnitus at time point 1 and time point 2 [188].

#### *Linear quantile regression model*

Linear quantile regression model was used in **Paper III** to analyze the relationship between exposure variables and hearing thresholds. Linear quantile regression accounts for the correlation between twin pairs and is less sensitive to deviation from a normal distribution of the response variable [189].

#### *Linear mixed models*

In **Paper II**, a linear mixed model was used to compare hearing thresholds and hearing threshold shifts respectively of tinnitus cases against non-tinnitus controls. The linear mixed model takes the correlation induced by the paired structure of twins into account [188].

#### *Incidence and Prevalence, and Age*

Incidence is the rate of new cases of tinnitus. It is reported as the number of new cases occurring within a period of 18 years (**Paper II**). Prevalence is the actual number of cases alive, with the hearing loss (**Paper I**) or with tinnitus (**Paper II**) at a particular date in time (point prevalence). Point prevalence only counts those alive on a particular date. Age is a confounding factor because it is associated with the hearing acuity (meaning that older people are more likely to have hearing loss), and it is also associated with the outcome (because older people are at greater risk of developing hearing loss and tinnitus) [190].

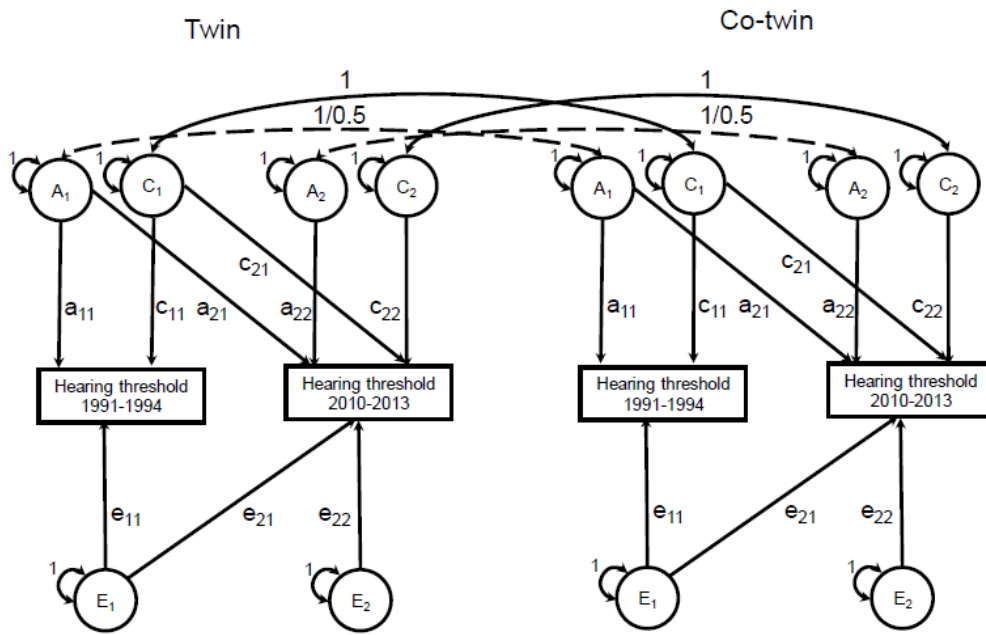
### *Indicators of twin similarity*

Identifying factors: similarities with birth dates, there is a connection linked in with the same birthday and shared genetic (100% MZ and 50% DZ); twins share some or all of the same common environmental factors before birth and during childhood or even later in life. The statistical analyses that assess twin similarity are as follows: probandwise concordant rate [191], tetrachoric correlations and structural equation modelling. Probandwise concordant rate was performed in MZ and DZ twin pairs to show the risk of having tinnitus given that co-twin has tinnitus. Tetrachoric correlation [192] was used to measure the similarity of twin pairs defined via the liability–threshold model (**Paper II**). Structural equation modelling see on page 28.

### *Twin correlations and heritability analyses*

The fact that monozygotic (identical) twins share all their genes and dizygotic (fraternal) twins share about 50% of their alleles, makes it possible to estimate what proportion of the phenotypic variation is due to genetic effects, shared environmental effects and unique environmental effects in twin studies. These three components are called A (additive genetic), C (common environment) and E (unique environment), and are used in the ACE (to estimate the relative influence of each factor on the total variance is  $A+C+E$ ). When identical twins are more similar than fraternal twins, then genetic influences are important for individual differences. When the identical twin similarity and fraternal twin similarity are the same, it is probably the shared environmental effects, such as family socialization and environment that affects the development of the outcome. Differences within identical pairs must be due to unique environmental influences.

The difference between the MZ and DZ correlation is due to a genetic similarity, and the additive genetic effects A is twice the difference between MZ and DZ correlation in **Paper I** and **Paper II**. Age distribution and follow–up time were shown between MZ, DZ and Singletons in **Paper I**. Intraclass correlation between twin pairs were used when comparing hearing thresholds in **Paper I**, and tinnitus status and hearing threshold shifts between tinnitus cases and controls at both time points in **Paper II**. The intraclass correlation is commonly used to quantify to what degree mono or dizygotic twins resemble each other in terms of a quantitative trait [192].



**Figure 6.** A path diagram of the bivariate longitudinal ACE model.  $A_1$ ,  $C_1$  and  $E_1$  correspond respectively to the genetic, shared environmental and non-shared environmental components that exist both at time point 1 and time point 2.  $A_2$ ,  $C_2$  and  $E_2$  capture respectively the genetic, shared environmental and non-shared environmental components that come into play during the follow-up. Each latent factor's relative strength on the hearing thresholds is indicated by the path coefficients. The path coefficients  $a_{11}$ ,  $c_{11}$  and  $e_{11}$  refer to the relative effects of latent factors on hearing threshold at time point 1. The relative strength of the path coefficients that retains at time point 2 are specified by  $a_{21}$ ,  $c_{21}$  and  $e_{21}$ . Subsequently path coefficients  $a_{22}$ ,  $c_{22}$  and  $e_{22}$  refer to the relative effect of latent factors that emerges during follow-up (Figure in Paper I).

### Structural equation modelling

Estimates of A, C, and E were derived by using structural equation modeling. One of the most important steps in structural equation modelling (SEM) is assessing whether a specified model fits the collected data or not. In this thesis, the SEM has been used to partition the total phenotypic variation into additive genetic (A), shared environmental (C), and individual-specific environmental variance (E). Nested models are created from the full model by constraining one or more of the parameters of the full model to zero to assess the contribution of the constrained parameters. Akaike Information Criterion and likelihood ratio chi-squared statistic were used to determine the significant difference between the full model and the nested model. The nested bivariate AE model was used, which indicate that removal of C (common environment) did not lead to any significant degradation of the model fit (C has no significance contribution to the model) in **Paper I** and **Paper II**.

Heritability is calculated as a proportion and shows if genes are the only reason for individual differences (1.0) or if genes do not contribute at all to phenotypic individual differences (0.0). Heritability, the proportion of the total variance attributed to additive genetic variance in the univariate model is computed by the ratio  $a^2/(a^2 + c^2 + e^2)$ . In the bivariate case (see Figure 6), it is obtained as the ratio  $a_{11}^2/(a_{11}^2 + c_{11}^2 + e_{11}^2)$  at time point 1 and at time point 2 as  $(a_{21}^2 + a_{22}^2)/(a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2)$  [192] (**Paper I** and **Paper II**).

### *Within twin pair analyses (co-twin control)*

To understand the role of environmental factors, the twin design is an excellent way by studying the differences within twin pairs. Identical twins share all their genes and also the common environment at the family-level, therefore, any differences reflect the unique environment. In this thesis, hearing thresholds were compared between twin 1 and twin 2 in both MZ and DZ twin pairs. Discordant comparison [192] with  $t$ -tests (one member of each pair belongs to an opposite classification, i.e. case or control) was calculated the difference between cases and controls at each frequency, threshold shifts and the correlation within twin pairs in **Paper II**, and a co-twin analysis [192] was performed in twin pairs discordant for a trait, i.e. one twin within the pair reported tinnitus and the other twin did not in **Paper II**. Twin pairs discordant for occupational noise exposure were used to analyze their hearing impairment, hearing thresholds at each frequency, and the difference in hearing threshold between right and left ear (**Paper III**).

The statistical analyses were processed using Stata 11.2, R 3.0.3. Open Mx 2.0. in **Paper I**; Stata 11.2, R 3.1.3 and Open Mx 2.2 in **Paper II** and Stata 11.2 and R 3.2.3 in **Paper III**.

## **3.5 ETHICS APPROVALS**

All participants at the start have received written information by letter about the study, that participation was voluntary and that they, whenever they so wished could cancel their participation. The participants signed informed consents at the appointment, and once more an oral information was given that participation was voluntary.

The studies, both in the early 1990's (Huddinge Hospital [18/92]) and in 2009 (2009/378–31) were approved by the Regional Ethical Review Board in Stockholm, Sweden.

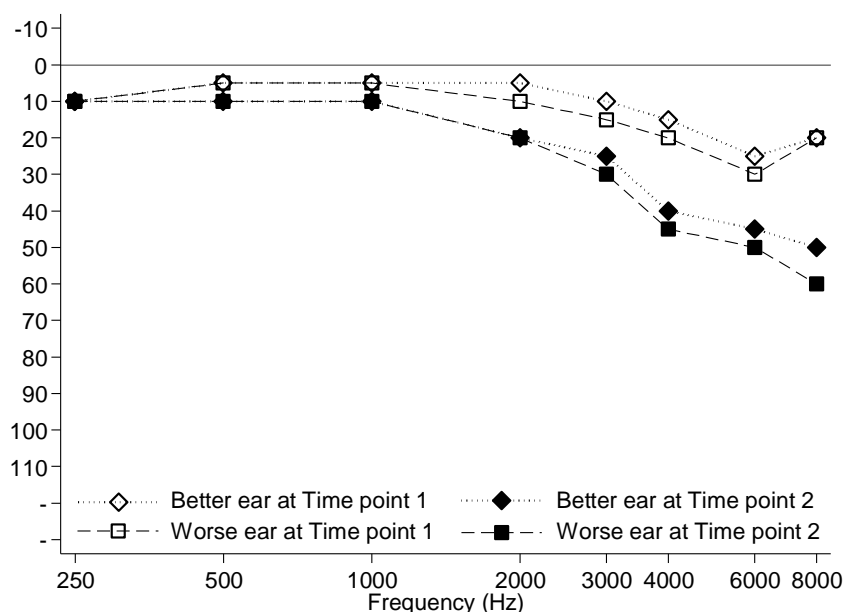
## 4 RESULTS AND COMMENTS

### 4.1 AGING, HEARING LOSS (PAPER I) AND TINNITUS (PAPER II)

The male participants ( $N = 1114$ ) at time point 1 were 34 to 79 years of age and 52 to 95 years of age at time point 2. This means that we investigated an aging cohort, in where two of the aims were to study the influence of aging on hearing loss and tinnitus.

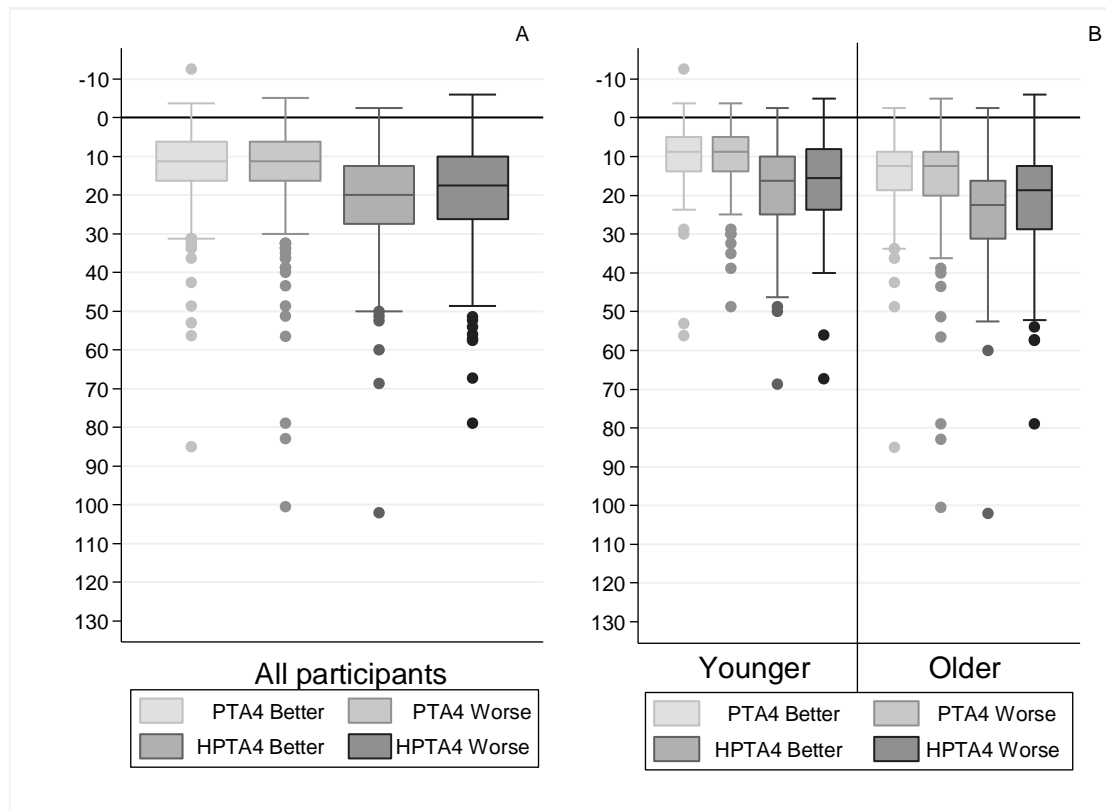
The results show that the prevalence of hearing loss in this cohort was increased by age and the hearing impairment increased during the follow-up period. The two main outcomes for hearing, the threshold averages PTA4 (500, 1000, 2000, and 4000 Hz) and HPTA4 (3000, 4000, 6000, and 8000 Hz), differ, and the high-frequency average has a higher prevalence. Comparisons were made after dividing the cohort into two age groups of equal size, younger and older than 66.4 years. Already at time point 1 the high-frequency average showed a predominantly higher prevalence for hearing impairment of 26% for the younger group and 65% for the older group (both for the worse ear), where the corresponding prevalence for the PTA4 was 5% and 22% (younger and older group for the worse ear)(see Paper I, Table 2)[183]. Two decades later, at time point 2, the prevalence had increased up to 69% for the younger group and 94% for the older group (worse ear) for HPTA4 whereas PTA4 had a prevalence of 26% and 59% for the younger and older group respectively.

The hearing thresholds at different frequencies were influenced by age and the mean audiogram ( $N = 583$ ) showed a sloping curve that corresponds to the typical audiogram of age related hearing loss, already at time point 1 especially in the worse ear (see Figure 7).



**Figure 7.** Median hearing thresholds of the twin cohort at time point 1 and time point 2 ( $N = 583$ ). Open symbols represent the measurements at time point 1. Closed symbols represent the measurements at time point 2.

The decline of hearing acuity over time for the whole cohort in PTA4 was 12.0 dB in the better ear and 12.3 dB in the worse ear. The HPTA4 showed greater threshold shifts across the 18-year follow-up: 21.1 dB in the better ear and 18.7 dB in the worse ear (Figure 8, Panel A). The decline of hearing over time, i.e. the threshold shift differed between the young and the old age group, where the older group had a greater threshold shift for both averages (PTA4 and HPTA4, see Figure 8, Panel B).



**Figure 8.** The difference between time point 1 and time point 2 measurements for PTA4 and HPTA4 in the better and worse ear for all participants ( $N = 583$ ) in Panel A and for the two groups, Younger ( $N = 288$ ) and Older ( $N = 295$ ) with a cut-off age 66.4 year (Panel B).

The hearing threshold shift was calculated as the rate of deterioration in decibels per year at all frequencies from 125 to 8000 Hz (Paper I, Table 1)[183]. The threshold shift for the younger group were under 0.5 dB per year from 125 to 2000 Hz, between 0.5 to 1.0 dB per year from 3000 to 6000 Hz, with the greatest shift at 8000 Hz with 1.3 dB per year deterioration (better ear). In the group of young participants the hearing deterioration in the worse ear was less than in the better ear even if it followed the same pattern over the frequency range. The older group on the other hand showed a more pronounced hearing deterioration in the worse ear up to 2000 Hz (0.9 dB per year at 1500 and 2000 Hz), and the deterioration above 2000 Hz ranged from 1.0 dB up to 1.6 dB, with the highest threshold shift at 8000 Hz (better ear: 1.6 dB per year and worse ear: 1.9 dB per year).



Tinnitus status was available for 1084 twins (missing data N = 30) at time point 1, showing the overall tinnitus prevalence of 13.5% ranging from 11.6% to 16.6%. Tinnitus status at time point 2 was available for 576 twins (missing data N = 7), where the tinnitus prevalence ranged from 29.7% to 37.4%, overall tinnitus prevalence 33.5%. Participants with tinnitus were generally older at time point 1, compared to non-tinnitus participants. Participants who reported tinnitus at both time points were also older than those who did not reported tinnitus at any time point. The hearing thresholds at all frequencies between 125 to 8000 Hz differed for twins who reported tinnitus and with those who did not have any tinnitus at either of the time points. Twins with tinnitus status had greater hearing thresholds.

Out of the 583 twins, there were 500 twins who reported no tinnitus at time point 1 and thereby were at risk to develop tinnitus at time point 2. The incidence proportion varied between 24.1–31.9% with an overall incidence proportion of 27.8%. Incidence Proportion was also calculated for the age group 50–59 years old (23.6%), 60–69 years old (32.4%) and 70 years old and above (23.5%). New tinnitus cases at time point 2 with the average age of 65.8 (64.6–66.9) had the greatest hearing threshold shift from 21.8 to 33.5 dB at 4000 to 8000 Hz, compared to the rest of the twins who were divided into other groups (namely: never reported tinnitus, reported tinnitus at both time points or reported tinnitus only at time point 1; see Paper II)[193]. The participants with tinnitus at time point 1 and at both time points were the oldest and also had the greatest threshold shift (9.5 dB–19.8 dB) from 125 Hz up to 2000 Hz (data from the better ear).

#### **4.1.1 Comments (Paper I and Paper II)**

Aging is a biological process affecting all cells in the body including in the auditory system. To study the slow gradual process, of aging demands a long follow-up period for any epidemiological study. The unique feature in this aging male twin cohort is a long follow-up period of two decades with audiometric and questionnaire data. Showing the influence of the aging process on hearing threshold was one of the main goals in this study. The results show typical decline on hearing threshold with aging and a sloping audiogram with the greatest loss at 8000 Hz confirms the results of other studies [21, 33, 38].

To compare the results of our study with other large epidemiological studies was a challenging experience. The age range in the twin cohort was already high at time point 1, resulting in an even higher age range twenty years later at time point 2. We found a statistically significant difference for the prevalence of hearing impairment, depending on which frequency average (PTA4 or HPTA4) that was studied. A study by Mościcki et al., (1985) had an 83% overall prevalence of hearing impairment for participants with an age from 57 to 89 [32]. It is higher than our overall prevalence, which can partly be explained by their different definition of hearing loss ( $\geq 20$  dB for the average of three frequencies 500, 1000, and 2000 Hz). Another cohort with participants between the ages of 48 to 92 years showed an overall prevalence for hearing loss (PTA4 average) of approximately 46% for the worse ear, showing an increasing prevalence with age [21]. In a Norwegian study by Borchgrevink et al., (2005) looking at PTA4 averages and showed a 22.2% (better ear) and

32% (worse ear) prevalence for hearing impairment for participants aged 20–101 years old [43].

All the three above mentioned studies used unscreened cohorts as we did, thus the most likely explanation to the varying higher prevalence values is the differences in ages and gender, especially in the Norwegian cohort.

Studies with self-reported tinnitus as an outcome are many, and the definition of tinnitus can vary between studies, which can influence the results. The type of study population also influences the results, depending on if the study is i.e. clinical or population based, gender specific or not, younger, older or mixed age cohort. The prevalence of tinnitus in the present study (Paper II) was 13.5% at time point 1 and 33.5% at time point 2 [193]. A study by Sindhusake and colleagues (2003) found similar tinnitus prevalence (37.2%) as in our study. Their population-based study was a cross-sectional unscreened for noise exposure, with both genders represented above 55 years of age, and the number of participants reached a bit over 2000 [35]. Nondahl et al., (2011) found a lower prevalence of tinnitus (10.6%) when they studied a younger cohort with both male and female with more than 3000 individuals [194]. The lower tinnitus prevalence compared with our results probably depends on the age of the cohort, but Nondahl et al., (2011) also used a more strict definition of tinnitus [27]. Another study by Nondahl and colleagues (2012) investigated the tinnitus prevalence in the generations born between 1925 and 1954. They found that tinnitus prevalence increases in the cohort representing those born around 1940 and later [25]. This result is similar to what we found in our study, which shows that new tinnitus cases were discovered among twins who were born in the 1940's and 1950's and was around 60 years old at time point 2 [193]. Finally, a recent study based on the Swedish Twin Registry reported a tinnitus prevalence of 14% for males, peaked to 25.1% in the age 65 to 74 years old [118].

The higher prevalence in our study can be due to on at least four reasons. First our cohort represented only males, secondly it was followed up after 18 years and was unscreened for different risk factors. Lastly, unfortunately the cohort lost almost half of the participants from time point 1. We may have underestimated the prevalence, as 21% (N = 112) of the dropouts were among the very old, that were unable to participate, and they probably would have had the worst hearing loss at time point 2. But there is a slight possibility that, we have overestimated the prevalence, since 79% (N = 419) of the dropouts were among the youngest in the study population, who probably had good hearing thresholds and thus were not interested in participation at time point 2.

Longitudinal studies help to understand the etiology of hearing deterioration over time. Often it takes at least 5 years before a hearing threshold shift can be detected, thus studies with more than 5 years follow-up time are adequate, but more than 10 years of follow-up time are desirable. Our male twin cohort was enrolled at time point 1, from year 1991 to 1995. The follow up period was almost 20 years (time point 2 from year 2010 to 2013). A study by Pedersen and colleagues (1989) followed a 70– years– old cohort (born year 1901 and 1906) up to their 80's and found an average deterioration on hearing threshold as 1.7 dB per year

from 1000 to 8000 Hz, but the most pronounced deterioration at 2000 Hz (2.5 dB per year) for the male participants [39]. When we compared this result with ours, we found a deterioration around 1 dB per year in our old male participants (Paper I Table 1)[183], and the highest deterioration at 8000 Hz (1.9 dB per year). The explanation for the difference between the two studies in the lower frequency range is that the cohort by Pedersen et al., (1989) was older. A study by Brant and Fozard (1990) estimated a rate of change during a 15-year follow-up period, and concluded that the deterioration rate are less before the age of 50 (0.3–0.4 dB per year) and much greater after the age of 50 (1.2–1.4 dB per year) for the tested frequencies (125 to 8000 Hz)[30]. Our study show a higher deterioration rate, which can be explained by our older cohort with only male participants and the longer follow-up period of almost two decades.

To gain knowledge for the etiology of the tinnitus symptom over time, there is a need for population-based studies with longer follow-up periods. The incidence for tinnitus in our study was 27.8%. Two research groups from Blue Mountain, Australia, and from Beaver Dam, United States, have focused on tinnitus in longitudinal studies. Their follow-up time was 5 years for Gopinath and colleagues (2010)[36] and 5 to 10 years for Nondahl and colleagues (2002, 2010)[23, 195]. Both groups investigated the incidence of tinnitus and found incidence rate of 5.7% [36] 12.7% and 18% [23, 195] and all studies also found that subjects with tinnitus had a poorer hearing compared with non-tinnitus participants. The difference between epidemiological studies which report incidence of tinnitus from low to high, depends on age distribution, study population size, gender differences and follow-up period. Our study had the longest follow-up period and only male participants, with an age range from 52 to 95 years, which affected our results.

## **4.2 RELATIVE IMPORTANCE OF GENETIC AND ENVIRONMENTAL INFLUENCES (PAPER I AND PAPER II)**

Results for genetic analyses of MZ and DZ twins show that genetic factors are of importance for hearing loss at time point 1 as well as at time point 2. The intraclass correlations were more than twice the size for MZ pairs (0.53) than they were for DZ pairs (0.22). The best fitting model was the AE model, where the individual-specific environmental influence (E) accounted for approximately 40% of the variance. The same genetic influences of importance at time point 1 (0.41–0.63) were also of importance at time point 2 (0.45–0.69), showing that no new genetic components came into play at time point 2. The yearly deterioration, measured as decibel per year, showed lower values for additive genetic influence (A) but much higher values for individual-specific environmental influences (E), thereby E is more important for the rate of change in hearing threshold and for the cross-sectional time point specific levels. The analyses also show that environmental influences appear to be greater for the rate of change in the better ear (0.88) than for the change in the worse ear (0.54).

Genetic influences for tinnitus were analyzed with probandwise concordant rates at both time point 1 and time point 2, which showed a much higher rate for MZ pairs compared with DZ pairs, indicating that genetic effects are important at time point 1 (MZ 0.46; DZ 0.07) and at

time point 2 (MZ 0.51; DZ 0.32). The tetrachoric correlation among MZ pairs were also consistently higher than among DZ pairs at time point 1 (MZ 0.46; DZ -0.04) and at time point 2 (MZ 0.47; DZ 0.10), which indicate that genetic factors (A) are of importance for the prevalence of tinnitus and also for the longitudinal continuity in tinnitus. Also for tinnitus the AE model fitted best, and the results from the model analyses indicated that unique environmental (E), not genetic influences were important for continuity in tinnitus over time. The proportion of additive genetic influences (A) for tinnitus was found to be almost the same at time point 1 (0.40) and at time point 2 (0.44). The genetic correlation for tinnitus between time point 1 and time point 2 was 0.51.

#### **4.2.1 Comments (Paper I and Paper II)**

A few other studies are estimating the genetic influences for hearing using different approaches, analysing families, siblings and twin pairs. The results from these studies show a wide range of heritability estimates, the variation mainly depending on the studies' population size, age, gender and family relatedness. Two studies are similar to our study focusing on aging twin pairs above 50 years of age with a comparable study size using MZ and DZ twin pair of the same gender. Viljanen et al., (2007) studied female Finnish twin pairs finding a wider but lower heritability estimate 22% on high-frequency average and the better ear. The lower heritability estimate can partly be explained by a more homogeneous sample than our male twins, since the female twins in the studied age range (63–76 years old) may not be exposed to noise in the environments or at work to the same extent as men are [113]. Wingfield et al., (2007) showed a heritability estimate for their male twin cohort of 67%, which is in comparison with our results, the small difference can be explained in age, as the United States twins were 52 to 60 years [112], whereas our Swedish twins were 34–79 (time point 1) and 52–95 (time point 2).

Three studies have estimated the heritability of tinnitus. The first study is a European multicentre study with 198 families, using family analyses and not twins, showed the heritability for tinnitus to be 16% [117]. The second study from Norway by Kvestad and colleagues (2010) reported heritability for tinnitus for the brothers 14% in a population-based cohort of 50000 individuals [116]. The differences in results from these two studies compared with our findings might partly be explained with differences in age span, and with the design use to calculate heritability. Maas and colleagues (2017) reported a cross-sectional heritability estimate of 41% in a Swedish study on male twins, similar for males as in our study. Maas could assess difference for unilateral vs. bilateral tinnitus, and there they saw a difference [118].

### 4.3 ENVIRONMENTAL EXPOSURES AND RISKFACTORS (PAPER III)

The result showed, that predominantly, 80% of the participants belonged to an exposure class with very low (between 75–85 dBA) or no noise exposure  $\leq 75$  dBA at work, and 20% belonged to an exposure class with high noise exposure  $\geq 85$  dBA at work. This distribution was the same at both time point 1 and time point 2. Age was a confounder and all statistical analyses for risk factors were adjusted for age. The median values for hearing thresholds at the worse ear in different exposure classes were presented in Paper III (Figure 1)[196]. The audiogram configurations showed relatively normal thresholds, better than 20 dB HL, from 125 up to 2000 Hz, but from 2000 Hz a steep slope with a highest threshold at 6000 Hz. The hearing threshold at 8000 Hz showed a better value than at 6000 Hz for all four noise exposure classes. The two groups that had the most different configurations of hearing thresholds on the audiograms were the reference class (without noise exposure) having the best hearing thresholds and the highest noise exposure class with the worst hearing thresholds at all tested frequencies at time point 1. This difference was statistically significant for the frequencies from 125 to 8000 Hz. On the other hand, at time point 2, only the group with the second highest noise exposure at work  $> 85$  dBA with 1 to 9 year of noise exposure, differed significantly from all other three groups. Both high and low–frequency averages (PTA4 and HPTA4) were affected by continual exposure to continuous occupational noise which was shown to be a risk factor at both time points.

Another environmental risk factor was the firearm impulse noise at leisure time that affected the high–frequency hearing thresholds at both time points, whereas solvent exposure only had a minor impact on the hearing thresholds. No statistically significant difference in hearing was found between smokers and non–smokers in this cohort. The most vulnerable twins were the small group (N = 31) who were exposed to both continuous noise at work above 85 dBA and to firearm use at leisure time. This group also had the statistically significant worse hearing thresholds. The results from the longitudinal threshold shifts showed that noise exposure at work  $\geq 85$  dB was a significant risk factor for a greater threshold shift during the follow–up time in participants who already had a hearing impairment (HPTA4  $\geq 25$  dB HL).

Risk factors for tinnitus were analyzed using GEE regression analysis, which took relatedness of the twin pairs into consideration. At time point 1, age had a significant odds ratio of 1.04 (95% CI 1.03–1.03,  $p < 0.001$ ), but age was a non–significant factor at time point 2 OR = 1.00 (95% CI 0.98–1.02,  $p = 0.934$ ). An increased risk for tinnitus was found at time point 1 in twins exposed to firearm noise at leisure time OR = 1.77 (95% CI 1.06–2.95,  $p < 0.05$ ), OR at time 2 was 1.57 (95% CI 0.94–2.92,  $p = 0.08$ ). At time point 2 an increased risk for tinnitus was also found for solvent exposure OR = 1.55 (95% CI 1.02–2.28,  $p < 0.05$ ). No type of occupational noise exposure was a significant risk factor for tinnitus.

Using twins that were discordant for occupational noise exposure (i.e. noise exposed  $> 85$  dBA and non–exposed for noise) the comparison between MZ and DZ twin pairs showed no statistically significant difference between the groups, even if the noise exposed twins have a higher hearing thresholds.

### 4.3.1 Comments (Paper III)

The result that lifetime occupational noise exposure affects the hearing is not surprising. A high frequency hearing impairment for individuals with noise exposure has been shown in several studies during many decades. In the late 1980's, Mościcki and colleagues (1985) showed that noise exposure for male participants ( $N \approx 560$ ) affected the high frequencies and the audiogram showed sharply decreased thresholds between 2000 and 4000 Hz [32]. Two published papers from the same Norwegian population-based cohort (HUNT) showed more elevated hearing thresholds for those who were exposed to noise [14, 44].

Noise exposure was highly correlated with the elevated hearing thresholds on the worse ear and associated also with the low-frequency average hearing level. Possible repeated high level of noise damage the sensitive hair cells during many years. This is causing hair cell loss in the cochlea starting the loss from the highest frequencies and when there no longer is an obstacle for the noise energy, middle and low frequency hair cells are the next target for the noise damage.

A major finding in our study was that individuals with an existing hearing impairment had an increased sensitivity to noise. A population-based Polish study by Kowalski et al., (2014) investigated a genetic variation playing a role in determining individual susceptibility to the development of NIHL, especially, in young adults and in those exposed to impulse noise and they concluded a cautious interpretation that a genetic variant may modify the susceptibility to NIHL development in humans [197]. A GWAS data by Grondin et al., (2015), showed a significant difference between the groups with and without hearing loss. Hearing thresholds were obtained before and after noise exposure, the hearing impaired group had worse hearing thresholds after noise exposure, in contrast, subjects performed similar in their hearing test before and after noise exposure [106]. Heinonen-Guzejev et al., (2005) have investigated the genetic component of noise sensitivity in their study. They concluded that self-reported noise exposure weakens the measured effect precision for noise sensitivity [119].

A significant finding in this study was that also lower noise levels 75–85 dBA can affect the hearing thresholds. This exposure is below the threshold limit values in Sweden and most other countries even if the Swedish and European Noise directive [176, 198] has implemented exposures above 80 dBA as a limit for the first action level. Our result strengthens the need for this action level and the need for hearing protection programs also at lower occupational noise exposure levels.

In our study, age is a known risk factor for tinnitus [193] and firearm use has also been shown to cause tinnitus by Shargorodsky et al., (2010)[139], thus these results are not surprising. Tinnitus is also connected to hearing loss which we have reported earlier [193, 199]. Firearm use was a significant risk for tinnitus and also a risk for hearing loss. It is thus not possible to differentiate if the effects on tinnitus are mediated through hearing loss or if this is a separate effect. Engdahl and colleagues (2012) reported an increased risk for tinnitus in participants

exposed to high levels of noise at work. This relationship was seen in 122 different occupations in almost 50000 subjects [200].

The pre-existing hearing impairment and the hearing loss during the 18 year follow-up time for both exposed and non-exposed discordant twin pairs confirms that individual specific environmental factors influence the hearing impairment. Our findings are similar to Wingfield et al., ( 2007) where the conclusion was that non-shared environment have an effect on both ears, and also genetic influences specific to different frequency range shown in the middle aged male twins [112].

## 5 CONCLUSIONS

- Age is, as could be expected, an important factor for both hearing loss (especially at high frequencies) and tinnitus prevalences at both time points
- Both tinnitus and hearing loss increase with age, an overall tinnitus prevalence of 33.5%, and in the oldest half of the cohort (> 66.4 years) the prevalence was as high as over 90% for high-frequency hearing loss. The overall incidence proportion was 27.8%, and the highest incidence proportion (32.4%) was for the age group 60–69 years old, while for the age groups 50–59 and 70 and above, the percentage was almost the same ( $\approx 23.5\%$ )
- Individuals with tinnitus have greater threshold shifts compared with those without tinnitus
- Genetic influences were important for both hearing loss and tinnitus. The influence had a greater effect on the lower frequencies and there was no difference in the degree of genetic influence at the two time points
- The hearing threshold shift, measured between time point 1 and 2, was influenced by the individual-specific environment. Thus environmental factors are important for the rate of change in hearing thresholds
- Firearm use at leisure time was a risk factor for hearing impairment and tinnitus, while no increased risk for hearing loss could be found for occupational solvent exposure or smoking
- Noise exposure was highly correlated with the hearing thresholds on the worse ear and associated also with the low-frequency average hearing level
- Early sign of hearing loss might increase the susceptibility to noise exposure
- Occupational noise exposure below the current threshold limit 85 dBA can affect hearing

## 6 GENERAL DISCUSSION

Age generally increased the prevalence for hearing loss and tinnitus. This is of importance for increased health care custody for the aging population, who requiring both healthcare and economic resources.

Both hearing loss and tinnitus had a moderate contribution of genetic influences, meaning that familial factors may be underlying the associations.

Regarding the environmental influences, we have looked at some factors and found the effects of some obvious ones, like noise and shooting but not by others like solvents and smoking. This can be explained by the fact that only a few twins have been exposed to solvents.

We found an increased noise sensitivity to already injured ears, which hopefully will mean a possible change in the risk assessments for noise exposure in working life with lower noise limit and/or shorter working hours in noise per day.

One of the major strengths is that it is derived from an 18-years longitudinal twin data. However, in this longitudinal study, we cannot exclude a certain amount of participants self-selection bias for attendance at the examination, where most participants are elderly, retired and has a pronounced hearing loss, and could provide systematic error [159]. It's possible that people's memory differ between time point 1 and time point 2 regarding recall of historical events. The questionnaire at time point 2 showed some contradictory self-reported information, but we adjusted for what was reported 20 years earlier, when the memories of events were more recent.

The main strength of this study is the use of a fairly large sample of same-sexed twin pairs, which provides the best framework of examining mono and dizygotic male twins, ensuring matches on age, sex, and shared familial factors [78].

Statistical power is a major concern in genetic association studies and this study is not an exception. The longitudinal approach caused a reduced sample size over time mostly due to natural causes. Thus some lack of statistical power due to small sample size has been an obstacle to perform more elaborate analysis involving model comparisons to single out the most significant contributing components to total variation in the structural equation models [78, 192].

### *Hearing measurement*

The audiometric data was collected by trained audiologists using sound isolated booths, earphones, audiometric equipment according to a standard clinical protocol.

PTA4 is the average that clinically is used to define hearing loss, or the degree of hearing loss in order to evaluate the individual need of rehabilitation. In the early years of 2000 WHO had a guideline for pure-tone average between 500–4000 Hz [185]. In our study we also wanted to explore the frequency region that reflects the age-related hearing loss (hearing loss above



2000 Hz) in a more equitable way, why an average of the higher frequencies 3000, 4000, 6000, and 8000 Hz (HPTA4) were calculated. The two averages (PTA4 and HPTA4) are both clinically used but could include different frequencies in other research studies. PTA4 provide a very careful depiction of hearing impairment. The PTA4 is not a measure that mirrors the onset of age-related hearing loss in early stage or the most adequate way, at least not for the most exposed males. A standard guideline for high-frequency average does not exist, at least not yet. If such standard were approved, it would have consequences on different levels, to exemplify, on personal, on professional, on a healthcare and primary level, and least on a governmental and financial level.

#### *Exposure estimate*

Occupational noise exposure was based on objective measurements from a validated job-exposure matrix [179] allocating occupational work code [180] to different work period of each individual. This was important in order to estimate the noise exposure during the entire working lifespan. The use of validated job-exposure matrix gave strength to our study as well, compared to other studies, where self-reported questions regarding noise exposure and self-reported hearing ability were asked [201, 202]. JEM is unbiased and gives an estimate on quantity based on observed data. In this study we used JEM for a quick and systematic way to classify coded job titles into a matrix of possible exposures for each separate job for each individual, thus making it possible to create individual lifetime noise exposure estimates [196]. A weakness of the study is that a lack of information in hearing protection use might bias the exposure estimation by somewhat overestimating the noise exposure. On the other hand collecting self-reported use of hearing protection over the lifespan of working life is almost impossible. This lack of information may open up for a risk of random misclassification, which may have biased the results in favor of the null hypothesis, but we believe that such a possible bias is small or moderate [159].

#### *Additional thoughts and future research*

In order to gain greater knowledge in hearing loss and tinnitus it would be ideal to include studies with extended family designs, where twins are linked with other family members, including children and grandchildren of the twins. The reason for this is that genetic factors are involved in both hearing loss and tinnitus. Future research provides a broader basis for understanding the mechanisms that affect hearing impairment. It would be valuable to investigate these variations more in future studies on larger twin cohorts.

Lastly, I would like to summarize, that our cohort study has captured two time points in the twin's lives.

The twins were born in the early 20<sup>th</sup> century and have been affected by the environmental impact and lifestyle of the 20<sup>th</sup> century, not neglecting the view of living conditions.

Genetic research begins its real revolution and our result of genetic impact on hearing is in its infancy.

We could definitely do more follow-up studies on this cohort, but we need to take dropouts into account due to the very high age of the oldest old. At least 5 to 7 years need to pass by in order to be able to detect a change in hearing thresholds with a 5 dB step measurement size. A follow-up study 2018 to 2020 with about 300 people would be highly desirable.

Regarding new studies in the future, one might use a different study design, with age groups as close to each other as possible and a follow-up every 5 years, for as long as possible.

Future genetic research will thus identify the risk factors more accurately regarding the genetic and environmental impact on hearing. This recent intensifying search seeks to open up new horizons for the hearing impaired to a positive outlook on life and on the working environment.

It is thought that people's mean age and general quality of life will increase. Hopefully our study can show the way and help future studies to better understand the hearing's multifaceted change in man.

Increased awareness and knowledge of the damaging effect of any noise exposure in the future may result in a more frequent health check-up on hearing, even in young age, and more frequent use of hearing protection. Last but not least, research efforts in the field of technology need to be mentioned regarding to the development of quiet running devices and everyday home products.

Our lives, be it private or professional, is very demanding and makes us forget about the importance of taking good care of ourselves. There are many stressors that may affect us either positively or negatively. These stressors may for example trigger tinnitus to full awareness in our mind thus affecting our daily life. We are only in the beginning of a phase when other studies are discovering more about the heritability of hearing and tinnitus and face a hunger for knowledge. Knowledge is important, as it is to talk about it, to pass it on, and to understand it.

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## 9 APPENDIX